

REGENERATION OF EXPERIMENTAL PERIODONTAL FURCATION DEFECTS

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Preface

This thesis is based on the following papers, which will be referred to by their Roman numerals.

- I. Klinge, B., Nilvéus, R., Kiger, R.D. & Egelberg, J. 1981. Effect of flap placement and defect size on healing of experimental furcation defects. Study 2. Dogs. Journal of Periodontal Research 16, 236-248.
- II. Klinge, B., Nilvéus, R. & Egelberg, J. 1984. Effect of periodic tooth displacement on healing of experimental furcation defects in dogs. Journal of Clinical Periodontology. Submitted for publication.
- III. Klinge, B., Nilvéus, R., Bogle, G., Badersten, A. & Egelberg, J. 1984. Effect of implants on healing of experimental furcation defects in dogs. Journal of Clinical Periodontology. Submitted for publication.
- IV. Klinge, B., Nilvéus, R. & Egelberg, J. 1984. Effect of crown-attached sutures on healing of experimental furcation defects in dogs. Journal of Clinical Periodontology. Submitted for publication.
- V. Klinge, B., Nilvéus, R. & Egelberg, J. 1984. Bone regeneration pattern and ankylosis in experimental furcation defects in dogs. Journal of Clinical Periodontology. Submitted for publication.

Abstract

An experimental model of through-and-through furcation defects was used to evaluate regenerative periodontal surgical procedures. Citric acid conditioning of the root surfaces was included in the treatment for all teeth. Variations of postoperative placement, suturing, and graft support of the flaps were investigated together with the relative importance of size of the defects and postoperative mobility of the treated teeth.

Successful healing of the defects was obtained following postoperative, coronal placement of the flap margins as opposed to flaps replaced at the approximate level of the cemento-enamel junction. Healing following replaced flaps could be improved by the use of crown-attached sutures to provide better postoperative wound closure and by the use of intraoral bone grafts to provide flap support.

Variations in the size of the defects seemed to be of minor importance to obtain new connective tissue attachment and closure of the defects. Also, postoperative periodic tooth displacement did not seem to interfere with the healing process.

Bone formation 9-10 weeks postoperatively seemed to be more advanced adjacent to the root surfaces than in the midportion of the interradicular area. Ankylosis was observed, and the incidence of ankylosis seemed to be higher in large defects than in small defects and also it seemed to increase with increased distance from the base of the original wound.

Introduction

In chronic periodontitis there is a gradual loss of the tooth-supporting tissues, eventually leading to loss of teeth. Traditionally, periodontal therapy has been aimed at arresting, rather than reversing this process. Ideal treatment should involve not only the prevention of further attachment loss, but also the complete regeneration of lost supporting tissues, comprising of new alveolar bone and a new periodontal ligament.

Regenerative attempts in humans

In clinical studies to date there has been limited success in achieving new attachment. The problems encountered seem to be related to flap management, preparation of the root surface and control of cells repopulating the treated root surface. A number of clinical studies have been performed evaluating factors and methods of enhancement of periodontal regeneration.

Importance of osseous defect morphology

The bone morphology of periodontal intraosseous lesions has been considered of importance in the context of obtaining regeneration following therapy. Enhanced regeneration has been observed as the number of osseous walls circumscribing the defects increases. 3-wall defects seem to show the most favorable response to treatment and 2-wall defects seem to have greater healing potential than 1-wall defects (Patur & Glickman 1962, Ellegaard & Löe 1971, Carraro et al. 1976, Quintero et al. 1982). However, other studies have demonstrated a similar amount of regeneration in 2-wall and 3-wall defects (Rosling et al. 1976a, 1976b, Polson & Heijl 1978).

The number of root surfaces involved by the osseous defect has also been considered as an influence on the healing response. Defects associated with one root surface only are thought to have a greater potential for re-

generation than defects encompassing two or three surfaces (Schaffer 1958, Wade 1966, Prichard 1972). However, Polson & Heijl (1978) did not observe any difference in osseous fill for defects associated with one or more root surfaces.

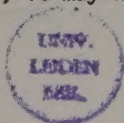
Epithelial exclusion

New connective tissue attachment is precluded if the cells from the oral epithelium proliferate along the root surface forming a long junctional epithelium. Björn et al. (1965) used a surgical technique whereby the epithelium was excluded from the root surface during healing. The crowns of the experimental teeth were cut off and the roots submerged under a mucosal tissue flap. The investigation demonstrated the formation of new cementum, periodontal ligament and alveolar bone. These results were confirmed in a similar investigation by Cook et al. (1977).

Recently, successful new attachment attempts have been made using membranes to prevent epithelium and dentogingival connective tissue from reaching the curetted root surface during healing. This technique allowed periodontal ligament cells to repopulate the wound area, so enhancing regeneration (Nyman et al. 1982b).

Effect of surgical debridement

Using clinical assessment criteria, regeneration of periodontal tissues can be obtained in man, following surgical debridement (Ellegaard & Löe 1971, Rosling et al. 1976a,b, Polson & Heijl 1978). The success or failure seems to be related to the presence or absence of bacterial plaque in areas where a regenerative procedure has been attempted (Rosling et al 1976a). However, histologic evaluation of healing after periodontal regenerative attempts in humans demonstrate that a long junctional epithelium may persist postoperatively along the root surface (Moskow et al. 1979, Listgarten & Rosenberg 1979, Steiner et al. 1981). Thus, it may not be likely that new



connective tissue attachment occurs following surgical debridement in man.

Effect of osseous grafts

Osseous grafts have been used as a method of enhancing regeneration following treatment of intraosseous defects. Reports on grafts of autogenous intraoral cancellous bone; autogenous or allogenic iliac cancellous bone and marrow, and lyophilized allogenic cortical bone have been published (Schallhorn et al. 1970, Schallhorn & Hiatt 1972, Dragoo & Sullivan 1973, Hiatt & Schallhorn 1973, Patur 1974, Mellonig et al. 1976, Sepe et al. 1978). These reports demonstrate successful healing following the use of each of these graft materials. However, in several studies where grafted lesions have been compared to non-grafted control sites the results are inconclusive (Carraro et al. 1976, Froum et al. 1976, Altieri et al. 1979, Pearson et al. 1981, Movin & Borring Møller 1982). In a review of the literature on 94 human specimens following bone grafting it was concluded that new attachment is more likely to occur when various grafts are used than in non-grafted sites (Bowers et al. 1982).

Effect of citric acid

In studies on periodontal wound healing, Register (1973) and Register & Burdick (1975, 1976) demonstrated enhanced connective tissue attachment and cementum formation to denuded root surfaces following conditioning of these surfaces with topical application of various acids. They concluded that citric acid, pH 1, applied for 3 minutes, was the optimal choice for regenerative attempts. Unequivocal new connective tissue attachment to root surfaces of human teeth previously part of epithelialized pocket was demonstrated in histological specimens by Cole et al. (1980). These results were confirmed by Albair et al. (1982). This is contrasted to the findings of Stahl & Froum (1977). They were unable to demonstrate histological evidence of new connective tissue attachment following citric acid conditioning.

Healing of acid-treated and non-acid treated periodontal defects was compared using clinical methods of evaluation (Cole et al. 1981, Renvert & Egelberg 1981). It was found that acid conditioning of the root surface resulted in a gain of probing attachment level which in statistical terms was significantly higher than the probing gain for non-acid treated controls. The clinical significance of the difference between acid treated and non-acid treated defects seemed to be limited.

Effect of tooth hypermobility

The effect of tooth hypermobility on the results of regenerative attempts has been evaluated by Rosling et al. (1976a). They reported that in treating infrabony defects all defects showed bone regeneration radiographically including defects adjacent to teeth that maintained hypermobility at the end of the 24-month observation period. These results were confirmed by Polson & Heijl (1978).

Fleszar et al. (1980), however, in analysis of data from an 8 year longitudinal study found that the change in level of probing attachment following periodontal therapy was related to initial degrees of tooth mobility.

Animal models to study periodontal regeneration

Intrabony defects

To evaluate various regenerative attempts experimental periodontal defects have been created in monkeys and dogs. Ellegaard et al. (1974) created 3-wall intrabony defects surgically in Rhesus monkeys. Spontaneous healing was prevented by steel bands or periodontal dressing left in place for 4 weeks. Of the 75 infrabony defects evaluated, 49 displayed signs characteristic of chronic periodontal lesions. In 20% of the 3-wall intrabony defects spontaneous healing occurred, possibly due to early loss of steel wires or periodontal dressings. Some spontaneous reattachment was observed in

the deeper parts of the remaining defects. Also, a tendency towards decrease of the width of the bony defects was observed.

Caton & Zander (1975) developed another Rhesus monkey model for testing periodontal treatment procedures. Defects were created by placement of orthodontic elastic bands around the teeth. Teeth with elastic bands in place during 12 weeks showed the typical histopathological features of periodontal pockets. Following removal of the elastics there was no evidence that either apical migration of epithelium or alveolar bone loss was reversible. The alveolar bone loss which had occurred was either horizontal or had an angular pattern. However, the angular defects seemed to be of limited magnitude. It was reported that almost identical lesions could be produced on contralateral teeth (Caton & Cowalski 1976).

Lindhe & Ericsson (1978) created periodontal breakdown by tying cotton floss ligatures around teeth at the cemento-enamel junction in beagle dogs. By changing the ligatures every 4 weeks and feeding the dogs a plaque promoting diet 1/3 of the supporting tissues was destroyed in a period of 25 weeks. Due to gingival recession however, the pockets remained shallow. Jansen et al. (1982) investigated the sequence of events that takes place in ligature induced periodontal defects following removal of the ligatures. The apical migration of the junctional epithelium was found to be irreversible. However, alveolar bone height tended to increase with time, indicating some bone repair.

Furcation defects

Ellegaard et al. (1973) induced through-and-through bifurcation defects in posterior teeth of Rhesus monkeys by passing a round bur through surgically denuded bifurcations. Spontaneous healing was avoided by placement of a steel wire or periodontal dressing in the created defects for 4 weeks postoperatively. The results indicate that chronic interradicular lesions with characteristics similar to periodontitis in man i.e. alveolar

bone loss, apical migration of the junctional epithelium and connective tissue inflammation were achieved. Johansson et al. (1978) used this experimental model for studies in dogs, reporting similar characteristics for this type of induced through-and-through furcation lesion and observing that the lesions remained epithelialized.

Regenerative attempts using the dog furcation model

The dog furcation model was utilized to evaluate the effect of autogenous cancellous bone grafts on healing of furcation defects by Nilvéus et al. (1978). The defects were surgically treated with and without adjunctive autogenous, cancellous bone grafts. Block specimens for histological analysis were removed after 8 weeks. All grafted teeth showed some reattachment after treatment. However, none of the specimens showed complete regeneration, as defined by new cementum formation all around the fornix of the furcation.

The effect of topical citric acid application on the healing of experimental furcation defects was evaluated in Labrador Retriever dogs (Crigger et al. 1978). In one mandibular quadrant the root surfaces were demineralized using citric acid with the other quadrant serving as a non-acid treated control. Histological evaluation showed a patent, epithelialized furcation for all non-acid treated teeth, whereas the majority of acid treated furcations showed new attachment. The significance of citric acid conditioning of instrumented root surfaces in achieving new attachment was confirmed in a later study in beagle dogs (Nilvéus et al. 1980).

The relative importance of systemic antibiotics, gelfoam as a matrix for blood coagulum, and coronal positioning of flaps was evaluated in 3 groups of beagle dogs following surgery that included citric acid conditioning of the root surfaces (Nilvéus & Egelberg 1980). A high rate of complete new attachment was obtained irrespective of the use of systemic antibiotics, gelfoam and flap positioning. Thus, topical citric acid treatment was again found to be the crucial therapeutic factor in achieving new attachment of

the experimentally induced furcation pockets.

Limitations of previous studies using the dog furcation model

The above mentioned dog furcation model utilized furcation pockets of limited size and without concomitant reduction of mesial and distal periodontal support. The new attachment obtained may, in part, be related to the limited defect size. Further studies of factors involved in the attainment of new attachment in furcation areas should use defects of larger magnitude.

Problems

Through-and through furcation defects in dog mandibular premolars with circumferentially reduced periodontal support was used as a model to study the following problems in periodontal regeneration:

1. What is the importance of postoperative positioning of the flap margins for successful healing of the defects?
2. Will flap support by graft materials or modified suturing techniques improve postoperative wound coverage and enhance healing?
3. What is the relative importance of size of the defects and postoperative mobility of the treated teeth?
4. What are the characteristics of interradicular bone regeneration?

Investigations and results

I. Effect of flap placement and defect size on healing of experimental furcation defects.

Inadequate postoperative flap coverage may counteract or minimize the beneficial effect of regenerative procedures. In previous studies using the dog furcation model, alveolar bone reduction has been limited to "key-hole" furcation defects without concomitant horizontal bone reduction. The high alveolar bone level proximal to the furcation may give better postoperative support to the flaps as compared to a situation where the bone is reduced circumferentially on the tooth. In the present study therefore, circumferential reduction of alveolar bone was utilized in the defect creation.

Through-and-through furcation defects were made in mandibular second, third and fourth premolars of 6 beagle dogs. Bone was surgically removed from the furcation and from the circumference of each tooth at 3 different levels. In 2 dogs, bone was removed to a level 2 mm apical to the cemento-enamel junction. 2 dogs had bone reduced 3.5 mm and another 2 dogs to a level of 5 mm from the cemento-enamel junction. Steel wires were passed through the furcations and ligated to enhance plaque formation and prevent spontaneous healing. At 6 weeks, these wires were removed. Reconstructive surgery was performed at 12 weeks and included reflecting mucoperiosteal flaps, debriding and planing the roots, and applying citric acid to the root surfaces. In 1 side of the mandibular arch the flaps were raised coronally to provide maximum coverage of the furcation wounds. On the other side of the jaw the flaps were replaced and sutured to the approximate level of the cemento-enamel junction. Each technique was alternated between the right and left side on successive dogs. The position of the gingival margin relative to the furcation fornix was measured throughout the study. 10 weeks after reconstructive surgery, block specimens were taken for histologic

evaluation.

Coronally positioned flaps allowed for an average gingival coverage of the furcations of 4.5 mm immediately after suturing. The flap margins gradually moved apically and after 1 to 2 weeks the location of the gingival margins corresponded to the most coronal point of the furcations. The replaced flaps allowed for an immediate average gingival coverage of 0.6 mm. These flap margins also receded apically and were generally positioned apical to the fornix of the furcations at the end of the experimental period.

Histological assessment of the healing showed that new attachment was achieved in 12 of 18 teeth following coronally positioned flap technique and in 1 out of 17 teeth following the replaced flap technique. The variations in defect depth encountered in the present study did not seem critical to the healing events as new connective tissue attachment occurred irrespective of defect size. Postoperative flap coverage of the furcation seems to be the critical factor for successful healing of furcation defects.

II. Effect of periodic tooth displacement on healing of experimental furcation defects in dogs.

The present study was performed to find out if healing of furcation defects, especially defects having a large vertical dimension, would be influenced by tooth hypermobility.

Furcation pockets were created in 6 dogs as described in Paper I. Bone was surgically removed from the furcation and around each tooth at 3 different levels: 3, 6, and 9 mm. Immediately following bone reduction the roots were planed and treated by topical citric acid application. Flaps were positioned coronally and sutured to provide maximum coverage of the furcation wounds. During the postoperative healing period the mandibular second, third and fourth premolars on 1 side were subjected to periodic displacement. Corresponding teeth on the contralateral side were splinted to serve as non-

mobile controls.

Stainless steel onlays extending from the cuspid to the first molar were fabricated for both sides of each mandible. In the experimental quadrants the onlays were cemented to the cuspid and first molar only, whereas all included teeth were splinted in the control quadrants. The onlays were perforated by circular holes over the premolars under study. On the experimental side these holes were adjusted to permit mesio-distal tooth displacement. Orthodontic elastics, fastened to hooks on the splints, were used to displace the teeth on the experimental side in a mesial or distal direction and the direction of tension alternated every day. 9 weeks after surgery, block specimens were removed, prepared for histologic evaluation and examined using the light microscope. Reattachment was observed in 12 of 14 displaced teeth and in 14 of 16 splinted teeth. There were no difference in amount of interradicular bone fill and incidence of ankylosis between displaced and splinted teeth. Variations of the vertical dimension of the defect did not seem to influence successful healing. Thus, periodontal healing of experimentally created furcation defects did not seem to be affected by tooth displacement following reconstructive surgery.

III. Effect of implants on healing of experimental furcation defects in dogs.

Extensive flap coverage using coronal positioning proved feasible in dogs: a similar procedure would be less applicable in humans. Therefore, this study was undertaken to determine if implants could, by supporting replaced flaps, substitute for coronally positioned flaps during healing and facilitate new attachment.

Defects with 3-4 mm circumferential bone reduction were created in 10 dogs as described in Paper I. During reconstructive surgery, 4 dogs received implants of autogenous intraoral cancellous bone, 2 of autogenous iliac cancellous bone and marrow, 2 of Proplast[®] and 2 of Avitene[®]. Flap coverage was

assessed throughout the postoperative period. 6 weeks after surgery, specimen blocks were removed and processed for histologic evaluation.

The average gingival coverage was 0.8 mm immediately after surgery for both implanted and control teeth. The flap margins gradually receded, and after a few weeks were generally at or below the fornix. At teeth treated with implants the gingival margins tended to recede less than at the control teeth. New attachment was observed in 6 of 12 teeth implanted with intraoral cancellous bone and in 2 of 6 teeth implanted with iliac bone; these 2 teeth also exhibited extensive root resorption and ankylosis. None of the 12 teeth implanted with Proplast® or Avitene® showed new attachment. Of the 29 teeth not receiving implants 28 became epithelialized. Our findings suggest that flap support by intraoral cancellous bone implants may facilitate new attachment. A more predictable technique, however, needs to be developed.

IV. Effect of crown-attached sutures on healing of experimental furcation defects in dogs.

This study was performed to test if a suturing technique aiming at improved adaptation and prolonged coverage of the furcation region following replaced flaps would favor the development of new attachment.

Defects with 3-4 mm circumferential bone reduction were created in 6 dogs as described in Paper I. Following reconstructive surgery the flaps were replaced and the sutures were bonded to acid etched enamel with a composite resin in 1 mandibular quadrant. As a control, on the contralateral side, the replaced flaps were sutured interdentally with single sutures. The position of the gingival margin relative to the fornix of the furcation was recorded throughout the study. Specimen blocks were removed 6 weeks after surgery.

The average position of the gingival margin immediately after surgery was 0.9 mm above the fornix in both crown-attached and single-sutured

groups. Flaps secured with crown-attached sutures retracted apically an average of 0.4 mm but still remained an average of 0.5 mm coronally to the furcations 6 weeks after surgery. 13 of the 14 teeth treated with the crown-attached sutures showed new attachment. Flaps secured with interdental sutures retracted apically to below the fornix of the furcation within 1 week after surgery and histologically all 10 furcations were found to be lined with epithelium. The results of this study indicate that replacement of flaps with crown-attached sutures favors new attachment. This suturing technique may be clinically applicable in humans.

V. Bone regeneration pattern and ankylosis in experimental furcation defects in dogs.

The material for this paper originated from two previous studies (Paper I and II). It included observations on 12 beagle dogs subjected to regenerative surgery of experimentally created furcation defects. Only specimens showing successful regeneration with reattachment in the full circumference of the furcation were included. Histometrics were employed to determine 1) the pattern of the interradicular bone formation; 2) the frequency and localization of ankylosis.

The extent of the interradicular bone apposition varied from limited amounts of bone regeneration to bone fill in the entire interradicular area. Typically, the bone formation was more advanced adjacent to the root surfaces than in the midportion of the interradicular area, thus creating a U-shaped pattern of the bone. Ankylosis was observed in 24 of the 39 specimens. It was found to be more frequent in large defects than in small defects. The incidence of ankylosis increased as bone formed more coronally. These findings suggest that coronal bone apposition may either require or be facilitated by the presence of a root surface and that undisturbed healing is dependent upon either cellular or some other activity originating in the periodontal ligament at the base of the wound.

Discussion

Acid conditioning

Reconstructive periodontal surgery of experimentally induced furcation defects using citric acid conditioning of the instrumented root surfaces was performed in the present studies. Acid treatment of a planed root surface will expose dentinal tubules and a network of collagen fibrils (Garrett et al. 1978, Daryabegi et al. 1981, Fernyhough & Page 1983, Lasho et al. 1983). The depth of the demineralization of root planed dentin following use of citric acid for 2-3 minutes amounts to 3-5 μm (Garrett et al. 1978). Electron microscopic observations indicate that the exposed collagen fibrils of the dentinal matrix interdigitate with newly formed collagen fibrils in the healing tissue after 7-14 days (Ririe et al. 1980). Polson & Proye (1983) suggested that a fibrin linkage to the exposed collagen fibrils is a precursor to the connective tissue attachment.

It has also been suggested that citric acid treatment of periodontally diseased roots can elute potentially toxic material from the root surfaces (Fine et al. 1980). Still others have suggested that citric acid provides a physico-chemical environment on the root surface that encourages cell adhesion and cell migration (Boyko et al. 1980, 1981, Fernyhough & Page 1983). It is possible that each of these mechanisms play a part in the enhancement of the connective tissue root attachment. Thus, acid conditioning following root planing seems to provide a hospitable surface for the critical biochemical and cellular healing events occurring in the coagulum immediately following wound closure.

Flap placement

In periodontal wound healing new connective tissue attachment is precluded if the periodontal lesion heals with reepithelialization (Karring et al. 1984). Thus, it seems important to exclude epithelium from proliferating

along the root surface. Coronal positioning of the flap margins following surgery may be helpful in such efforts. Following use of coronally positioned flaps in the present studies a majority of teeth showed new attachment and complete closure of the defects. This was contrasted with an absence of new attachment following replacement of the flaps near the cemento-enamel junction. Following coronally positioned flaps epithelium is most likely excluded from the early healing events and new connective tissue attachment may be accomplished, whereas following a replaced flap procedure the flap margins will recede sooner and epithelium from the gingival margin will migrate along the root surface before connective tissue attachment has been established.

Gingival recession of replaced flaps could be reduced with crown-attached sutures. This suturing technique resulted in new connective tissue attachment. The distance from the gingival margin to the furcation fornix was less following crown-attached sutures than following coronally positioned flaps. The successful healing response following both techniques, however, indicate that the coverage achieved with crown-attached sutures is sufficient for new attachment to occur. This may indicate that a limited flap coverage is sufficient of establishment of new attachment, if the flaps stay stable during the early events of wound organization. These findings seem to concur with the report by Polson & Proye (1983) on early healing events following replantation of demineralized roots. Connective tissue attachment to the root surface appeared to be dependent upon a healing sequence related to fibrin and collagen interactions taking place within the first few days after replantation. By providing a stable wound healing support and preventing rupture of the delicate initial fibrin and collagen interactions, new connective tissue attachment may be achieved.

Use of implants

Implants for support of replaced flaps to prolong gingival coverage of

the root surfaces and thus facilitate new attachment was shown to be of limited value. The use of Proplast® and Avitene® did not result in new attachment. These materials may not have been incorporated into the healing process in the furcation prior to epithelialization. New attachment occurring following implantation of fresh autogenous iliac cancellous bone and marrow was accompanied by root resorption and ankylosis. Ellegaard et al. (1973, 1974, 1976) and Adell (1974) also observed root resorption and ankylosis following transplantation of fresh bone marrow. It is not known whether the grafted bone marrow cells are directly involved in the resorptions and ankylosis due to their high osteogenic potential, or if the graft induces these aberrations by an action on the regenerating periodontal tissues.

New attachment was achieved in half the number of defects following implantation of intraoral cancellous bone. Successful healing was associated with maintained flap coverage. A biocompatible implant like autogenous cancellous bone that is integrated early in the healing process may act as a mechanical flap support and thereby facilitate new attachment. Also, tissue stimulating factors may be innate in various implants.

Defect size

Variation of the vertical dimension of the defects appears to play a secondary role in the present dog model. Using the coronally positioned flaps, new attachment was achieved irrespective of defect size. Thus, it seems that once postoperative flap coverage is established and maintained during the critical postoperative phase, new connective tissue attachment can be achieved.

Tooth hypermobility

Tooth displacement did not seem to affect the interradicular healing events; defects of large vertical dimension included. The magnitude of tooth displacement, however, may have been less in this study than that of

Lindhe & Ericsson (1976), who demonstrated impaired healing following jiggling type trauma from occlusion. Thus, the tooth displacement in the present study may have been too limited to influence periodontal regeneration.

Bone regeneration pattern

During healing of the furcation defects bone regeneration seems to occur first adjacent to root surfaces, followed by a subsequent fill of the central portion of the interradicular area. This phenomenon suggests that coronal bone apposition may require or be facilitated by the presence of a root surface. Alternatively, it may indicate that coronal bone growth is dependent upon cellular or some other activity originating in the periodontal ligament.

During coronal regeneration of the interradicular tissues, the periodontal ligament space had frequently been obliterated by ankylosis of bone to the root surface. Melcher (1970, 1976) suggested that activity by cells originating from the intact periodontal ligament may be a prerequisite for the regeneration of new normal periodontal ligament and that regeneration of periodontal ligament does not appear to be as aggressive as that of alveolar bone. This concept has gained support from several studies (Atrizadeh et al. 1982, Karring et al. 1980, 1984, Line et al. 1974, Nyman et al. 1980, 1982a). In the furcation defects, ankylosis was more frequent in large defects than in small defects. The incidence of ankylosis increased as bone was forming more coronally. Thus, it can be speculated that ligament progenitor cells migrating coronally and forming a periodontal ligament are "overrun" by bone forming cells and this occurs more readily as the distance from the base of the wound increases.

Minor areas of root resorption were often observed. The majority of the resorptions showed "repair" with a cementum-like substance or with bone ankylosed to the root surface. This repair, however, may be temporary. It is possible that these small areas of resorption continue to develop and may

cause extensive replacement of root structure at a later time point.

Considerations for future studies

In the present series of studies, citric acid conditioning of root surfaces was used and resulted in connective tissue attachment in extensive furcation defects. New attachment following acid conditioning has also been achieved in dogs with naturally occurring periodontal disease and in human periodontal lesions (Bogle et al. 1981, 1983, Cole et al. 1981). Recently, techniques with placement of membranes to wall off epithelium and gingival connective tissue during the primary healing phase have shown to promote new attachment (Nyman et al. 1982a, Nyman et al. 1982b, Aukhill et al. 1983). Thus the attainment of new attachment is a biological reality. However, root resorptions have been observed in some studies (Aukhill et al. 1983, Bogle et al. 1984, Magnusson et al. 1984). By further development of techniques allowing for selective cell repopulation it may be possible to overcome this unwanted sequelae to periodontal regeneration.

Conclusions

The following conclusions can be made from these studies on healing of experimentally induced through-and-through furcation defects in beagles following reconstructive periodontal surgery using citric acid conditioning of the instrumented root surface:

1. coronal positioning of the flap margin facilitates the attainment of new attachment;
2. replaced flap procedures with conventional suturing techniques and without the use of supplementary grafting procedures do not provide new attachment;
3. the use of intraoral cancellous bone grafts to provide flap support for the replaced flaps may facilitate new attachment;
4. the use of iliac crest cancellous bone and marrow grafts may facilitate new attachment following replaced flaps but seems undesirable to use due to the development of root resorption;
5. the use of xenografts (Avitene® and Proplast®) does not facilitate new attachment following replaced flaps;
6. the use of crown-attached sutures to improve postoperative wound closure following replaced flap facilitates new attachment;
7. variations in the size of the defects seem to be of minor importance in obtaining new attachment;
8. experimental tooth displacement does not seem to interfere with the formation of connective tissue attachment;
9. bone formation 9-10 weeks postoperatively seems to be more advanced adjacent to the root surfaces than in the midportion of the interradicular area;
10. the incidence of ankylosis seems to be higher in large defects than in small defects and it seems to increase with increased distance from the base of the original wound.

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Paper I

Effect of flap placement and defect size on healing of experimental furcation defects

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Through-and-through furcation defects were experimentally created in one group of monkeys (Study 1) and in one group of dogs (Study 2). The effect of reconstructive periodontal surgery using citric acid conditioning of the instrumented root surfaces was studied. Two experimental variables were investigated: (1) variation of the placement and suturing of the flaps and (2) variation of the size of the furcation defects.

The results of these studies indicate that adequate postoperative flap coverage of the furcations seems to be critical for successful healing of the furcation defects. Variations in the vertical dimension of the defects seems to play a secondary role. Future studies in experimental animals should evaluate methods which will provide improved post-operative coverage of the furcation area during healing of furcation defects in human patients.

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Introduction

Studies in animal models are useful for investigating the effect of therapeutic measures which could facilitate regeneration and new attachment in periodontal furcation defects. Ellegaard et al. (1973) induced through-and-through bifurcation defects in posterior teeth of Rhesus monkeys by passing a round bur through surgically denuded bifurcations. Spontaneous healing was avoided by placing a steel wire or periodontal dressing in the created defects during a four week post-operative period.

Johansson, Nilvéus and Egelberg (1978) adopted the experimental model of Ellegaard et al. (1973) for studies in dogs and investigated the therapeutic effect of autogenous bone grafts. Through-and-through furcation defects were created in mandibular

premolars and were surgically treated with and without osseous grafts from healing extraction sites in the maxilla. The results of this study showed first that surgical debridement (removal of gingival epithelium and connective tissue from the interradicular region, thorough instrumentation of the root surface, and careful coronal positioning of the flaps) did not lead to new attachment in the dog furcation model and second that grafting of newly formed bone from extraction sites to the furcation was not successful (Nilvéus, Johansson & Egelberg 1978).

In subsequent investigations the effect of citric acid conditioning of the instrumented root surfaces as a supplement to surgical debridement was studied using the dog model (Crigger et al. 1978, Nilvéus et al. 1980, Nilvéus & Egelberg 1980). All of the non-acid

treated control teeth revealed a patent, epithelialized furcation defect after treatment, whereas the majority of the acid-treated furcations showed new attachment with new cementum formed along the full circumference of the furcation.

Ultrastructural studies by Ririe, Crigger and Selvig (1980) and Selvig et al. (1980) suggest that the improved healing following citric acid conditioning is related to demineralization of the dentin surface and exposure of collagen fibrils in the root dentin. During healing, these exposed fibrils of the dentin matrix seem to interdigitate with the newly formed collagen fibrils of the soft tissue flap. This mechanism may be the cause of the accelerated healing and connective tissue attachment to the acid conditioned tooth as compared to the non-acid treated root surface.

Our previous studies on the effect of citric acid conditioning have been performed in dogs. The peculiar anatomy of the teeth and supporting tissues in the premolar regions of the dog facilitates surgical management and flap closure during surgery (Nilvéus et al. 1978). In the monkey the anatomy in the mandibular premolar and molar region is similar to the corresponding human dentition. In the monkey, as in humans, adequate post-operative flap coverage of the furcation defects may be difficult to achieve. Inadequate flap coverage may counteract or minimize the beneficial effect of citric acid conditioning. The aim of the first of the present two studies, therefore, was to investigate whether citric acid conditioning leads to new attachment in experimentally created through-and-through furcation defects in monkeys as it does in dogs.

Study 1. Monkeys.

Material and Methods

Animals

Five female adult pig-tailed macaques (*M.*

nemestrina) were used. Their weight ranged between five and six kilograms. The mandibular second premolars, first and second molars (P2, M1, M2) were subjected to experimentation. All operative procedures were carried out under intramuscular injected Ketamine Hydrochloride anesthesia (Vetalar®, Parke-Davis & Co., Detroit, Michigan, USA), with atropine sulfate to reduce salivation (Atropine Sulfate, Eli Lilly & Co., Indianapolis, Indiana, USA). The animals were fed primate chow (Lab Chows® Ralston Purina Company, St. Louis, Missouri, USA) ground to grainy consistency during the entire experimental period.

Induction of Defects

Chronic, through-and-through furcation defects were created using a modification of the method of Ellegaard et al. (1973).

Following infiltrative anesthesia for hemostasis, full thickness buccal and lingual flaps were raised to expose the surgical site. As determined by pre-surgical decision (see Table 1) one side of the mandibular arch was subjected to creation of a "key-hole" furcation defect where bone removal was limited to the furcation area to the extent that permitted instrumentation with a small

Table 1

Study 1. Monkeys. Distribution of experimental procedures

Monkey #	Mandibular Quadrant					
	Right			Left		
1	M2*	M1	P2	p2	m1	m2*
2	m2	m1	p2*	P2*	M1	M2
3	M2	M1*	P2	p2	m1*	m2
4	m2	m1*	p2	P2	M1*	M2
5	M2	M1	P2*	p2*	m1	m2

* = non-acid treated controls

p2, P2 = second premolar

m1, M1 = first molar

m2, M2 = second molar

Small letters (p2, m1, m2) represent "key-hole" defects
Capital letters (P2, M1, M2) represent defects with reduction of proximal bone support

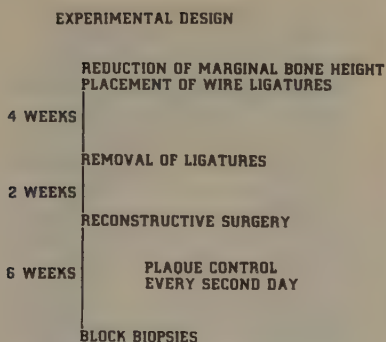


Fig. 1. Study 1. Monkeys. Experimental design.

hand chisel through the denuded fornix. On the opposite mandibular quadrant bone was surgically removed from the furcation area as well as from the full circumference of the tooth, creating a "horizontal" pattern of bone loss involving the furcation area and proximal bone to a level of 3-4 mm apical to the cemento-enamel junction. This bone removal was again accomplished with a small hand chisel. Steel wires were ligated through the furcation and over the occlusal surfaces and the flaps were then sutured with 4-0 silk sutures. These ligatures were left in place for four weeks after surgery, removed, and the defects were left on additional two weeks after ligature removal before reconstructive surgery was done. The experimental design is presented in Figure 1.

Experimental Procedures

The reconstructive surgery included elevation of mucoperiosteal flaps, debridement, root planing and topical citric acid application (Crigger et al. 1978, Nilvéus et al. 1980). One tooth in each quadrant operated was used as a non-acid treated control (Table 1).

The flaps were replaced and secured with

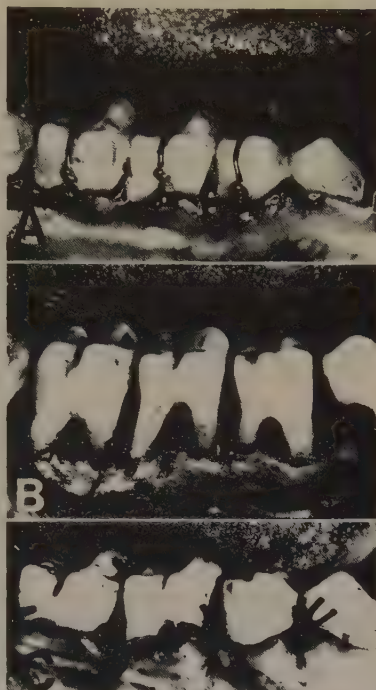


Fig. 2. Study 1. Monkeys. Right mandibular arch of a monkey subjected to reduction of the horizontal level of the alveolar bone.

A. Conditions four weeks after initial induction of defects, immediately before removal of steel wires.

B. At reconstructive surgery demonstrating the alveolar bone height.

C. After suturing of the flaps.

single interproximal sutures as coronally as possible (Fig. 2). The position of the flap margin in relation to the furcations on the buccal aspects of the teeth was recorded. First, the distance (A, see Fig. 3) between the most coronal point of the furcation fornix and a groove made into the buccal aspect of the central occlusal fossa was measured during surgery using a compass. Subsequently, the distance (B) between the gin-

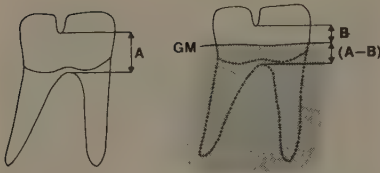


Fig. 3. Study 1. Monkeys. Illustration of the method of measurement of the extent of post-operative gingival coverage of the furcations. A = distance between the most coronal point of the furcation fornix and a groove made into the buccal aspect of the central occlusal fossa. B = distance between the gingival margin and the occlusal groove. A-B = extent of gingival coverage of the furcation. GM = gingival margin.

gingival margin and the same groove in the buccal aspect of the central fossa was recorded after suturing of the flaps. By subtracting the recorded distance for (B) from the distance for (A) the extent of gingival coverage of the furcation apertures was obtained (A-B, see Fig. 3).

Plaque control was instituted by tooth-brushing with a 2 % chlorhexidine solution (Hibitane®, ICI Ltd, Macclesfield, Great Britain) every second day for the full experimental period. The plaque control was carried out under brief, light ketamine anesthesia.

Histological Evaluation

Six weeks after reconstructive surgery, the animals were sacrificed by perfusion with formalin through the carotid arteries. Specimen blocks consisting of tooth, bone and soft periodontal tissues were then removed. The blocks were postfixed in formalin decalcified in formic acid, trimmed and embedded in paraffin. Serial, 7 μm thick sections in the mesio-distal plane were cut throughout the entire bucco-lingual extension of the teeth. Step serial sections at 98 μm intervals (every 14th section) were stained with hematoxylin and eosin and ex-

amined with the light microscope. The following assessments were made:

1. Proportion of teeth with new attachment in the furcation area according to the following criteria:

Complete new attachment: connective tissue regeneration including the formation of new cementum in the full circumference of the furcation in at least three consecutive step serial sections at 98 μm interval (bridging a total span of 196 μm).

Incomplete new attachment: connective tissue regeneration in the full circumference of the furcation in at least three consecutive step serial sections but lack of visible new cementum in part of the circumference of the furcation or specimens demonstrating area of ankylosis in one or more of the three sections.

Epithelialized furcation: presence of an epithelial lining in the furcation fornix (three consecutive step serial sections to satisfy the criteria of complete or incomplete new attachment were not found).

2. Interradicular bone level. All step serial sections at 98 μm intervals were examined under 40 X magnification. First, the distance between a line through the most coronal point of the furcation fornix and a line tangential to the most coronal part of the interradsicular bone was measured (C in Fig. 4). The section with the shortest distance and the adjacent step serial sections buccal and lingual to this section representing a span of 196 μm were selected. The mean bone level of these three selected sections was then expressed in relation to the mean level of the apical termination of root planing for the same sections ($\frac{A+B}{2} - C$ in

Fig. 4).

3. Defect depth. The three step serial sections selected for interradsicular bone lev-

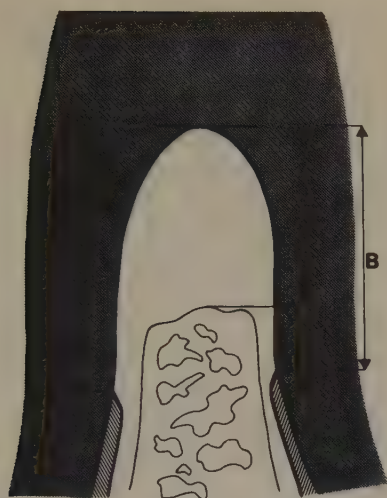


Fig. 4. Study 1. Monkeys. Illustration of histologic measurements of defect depth and interradicular bone level. A, B = perpendicular distance between the most coronal point of the furcation fornix and the apical termination of root planing for mesial and distal root surfaces. C = perpendicular distance between the furcation fornix and the most coronal part of interradicular bone. Defect depth = $\frac{A+B}{2}$. Interradicular

bone level = $\frac{A+B}{2} - C$.

el determination were used to measure the depth of the furcation defects at the

time of reconstructive surgery. The distance between the most coronal point of the furcation fornix and a line tangential to the apical termination of root planing was measured for the mesial and distal root surfaces of the furcation area. The average of the mesial and distal measurements for the three sections was used to compute the mean defect depth of each tooth ($\frac{A+B}{2}$ in Fig. 4).

Results

The results of the histological assessment of the healing of the furcation areas are presented in Table 2. New attachment (complete & incomplete) was achieved in 9 of the 20 teeth following citric acid conditioning. All of the ten non-acid treated control teeth demonstrated an epithelialized furcation fornix.

Fig. 5. presents the histological healing response in relation to the defect depth at the time of reconstructive surgery. No apparent relationship could be seen between defect depth and healing response. Therefore, defect depth seemed to have a limited influence on the success of treatment. All non-acid treated controls became epithelialized regardless of defect size. Some medium-sized acid-treated defects showed new

Table 2

Study 1. Monkeys. Distribution of histological healing response. Number of acid-treated teeth and non-acid treated controls showing complete new attachment, incomplete new attachment and epithelialized furcation

	Complete New Attachment	Incomplete New Attachment	Epithelialized Furcation
Citric acid treatment (N = 20)	3	6*	11
Non-acid treated controls (N = 10)	0	0	10

* 2 of these specimens showed ankylosis.

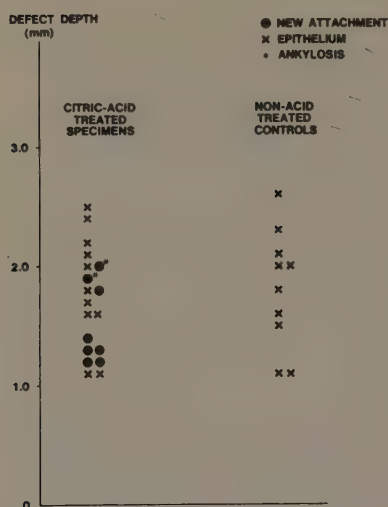


Fig. 5. Study 1. Monkeys. Histological healing response in relation to defect depth at the time of reconstructive surgery for citric acid treated specimens and for non-acid treated controls. Healing with new attachment in this graph includes specimen demonstrating complete as well as incomplete new attachment. (One specimen excluded from analysis of defect depth.)

attachment while few small acid-treated lesions became epithelialized.

Fig. 6 summarizes the histological healing response in relation to the extent of gingival coverage of the furcations at the completion of surgery. The gingival coverage of the furcations ranged between a location of the gingival margin 2 mm coronal to the fornix of the furcation to a lack of coverage evidenced by a location of the gingival margin 1 mm apical to the furcation fornix. Non-acid treated controls became epithelialized regardless of the amount of flap coverage. The acid-treated specimens that demonstrated new attachment showed an approximate gingival coverage of 0.5–1.0 mm.

The mean interradicular bone level for

the non-acid treated control teeth, all epithelialized, was 0.11 mm coronal to the apical termination of root planing. The acid conditioned teeth showed a bone level 0.95 mm coronal to apical termination of root planing for the teeth where new attachment was obtained and 0.16 mm for the acid-conditioned teeth which became epithelialized.

Areas of ankylosis were observed in two of the nine teeth which demonstrated new attachment. The two teeth with ankylosis

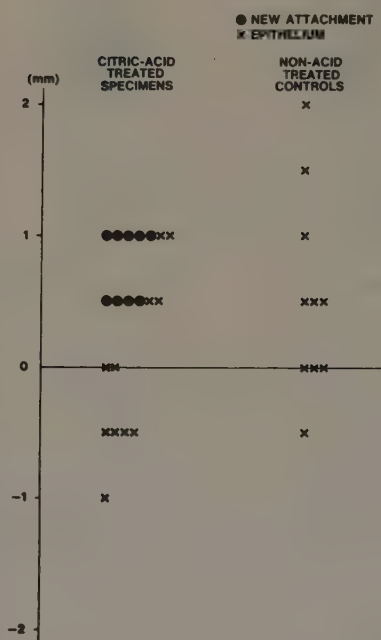


Fig. 6. Study 1. Monkeys. Histological healing response (complete & incomplete new attachment versus epithelium) in relation to the extent of gingival coverage of the furcations at the completion of surgery. Negative numbers signify a location of the gingival margin apical to the fornix of the furcation.

had the largest defect depth of the nine specimens with new attachment (Fig. 5).

Discussion

Reconstructive periodontal surgery of experimentally induced through-and-through furcation defects in monkeys using citric acid conditioning of the instrumented root surface was performed in the first of the present two experimental studies. Non-acid treated teeth served as controls. The results indicate that acid conditioning seems to be a prerequisite to achieving new attachment in the present monkey model. This finding is in agreement with our previous results in dogs (Crigger et al. 1978, Nilvéus et al. 1980, Nilvéus & Egelberg 1980). The frequency of successful closure of the furcations seems to be lower in the monkeys than previously observed in the dogs.

The variations in defect depth encountered in the present study did not seem critical to the healing events. New attachment after citric acid conditioning seemed unrelated to the defect size.

The initial reduction of proximal alveolar bone on one side of the animals was performed in order to study the possibilities of obtaining good post-operative flap coverage of the furcation areas. The results of the study seem to suggest that difficulties in achieving satisfactory flap closure over the furcation area may result in a lower healing response.

Because of the limited material in the present study, no definite conclusions can be drawn about the importance of post-operative flap coverage or defect size. But this study would seem to suggest that coverage of the furcation by the flap may be an important factor in generating new attachment. An additional study, therefore, was performed to focus on the significance of these two factors during healing of furcation defects. Dogs were chosen for this study

because of the ability for varying and controlling the post-operative flap coverage in our experimental dog model (Crigger et al. 1978, Nilvéus et al. 1980, Nilvéus & Egelberg 1980).

Study 2. Dogs.

Material and Methods

Animals

Six male beagle dogs were used, 15–24 months old and weighed 9–15 kilograms. The mandibular second, third and fourth premolars (P2, P3 & P4) were subjected to experimentation. All operative procedures were carried out under intravenous pentobarbital sodium anesthesia (Mebumal Vet, ACO, Sweden). The animals were fed a soft diet (Skippy® C.B.H. Foods Inc., Terminal Island, California, USA) during the entire experimental period.

Induction of defects

Chronic, through-and-through furcation defects were created using a modification of the method of Johansson et al. (1978). The modification involved a reduction of the horizontal alveolar bone level by different amounts. Bone was surgically removed from the furcation area and around the full circumference of the tooth including the prox-

EXPERIMENTAL DESIGN

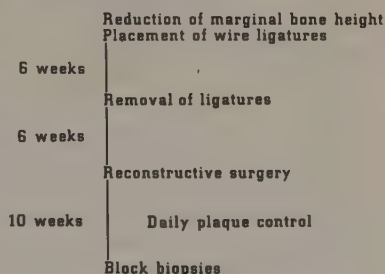


Fig. 7. Study 2. Dogs. Experimental design.

imal bone. Three different levels of bone reduction were studied. In two dogs bone was removed to a level 2 mm apical to the cemento-enamel junction. Two dogs had bone reduced 3.5 mm and another two dogs to a level 5 mm from the cemento-enamel junction. Steel wires were ligated through the furcation defects and removed after six weeks. Six weeks later reconstructive surgery was performed (Fig. 7).

The alveolar bone level was measured at six defined points around the circumference of the teeth at the time of initial osseous reduction and at reconstructive surgery (distal, distal-buccal, disto-lingual, mesio-buccal, mesio-lingual and mesial). The mean of these six measurements was calculated to show the bone level for each tooth. These recordings were performed to evaluate the changes of bone morphology and to provide information on the actual bone level at the time of reconstructive surgery.

Experimental Procedures

The reconstructive surgery included elevation of mucoperiosteal flaps, debridement, root planing and topical citric acid application. In one side of the mandibular arch the flaps were raised coronally to provide maximum coverage of the furcation wounds as previously described by Nilvéus et al. (1978) and Crigger et al. (1978). On the other side of the jaw the flaps were replaced and sutured to the approximate level of the cemento-enamel junction (Figs. 8 and 9). The coronally positioned and replaced flap techniques were alternated between left and right sides. The position of the gingival margin in relation to the furcations was recorded on the buccal aspects of the teeth throughout the post-operative period as described in Study 1. Of the 18 teeth with replaced flaps, one tooth was lost during the post-surgical period due to inadequate bone support leaving 17 teeth for analysis.

Plaque control was instituted by daily top-

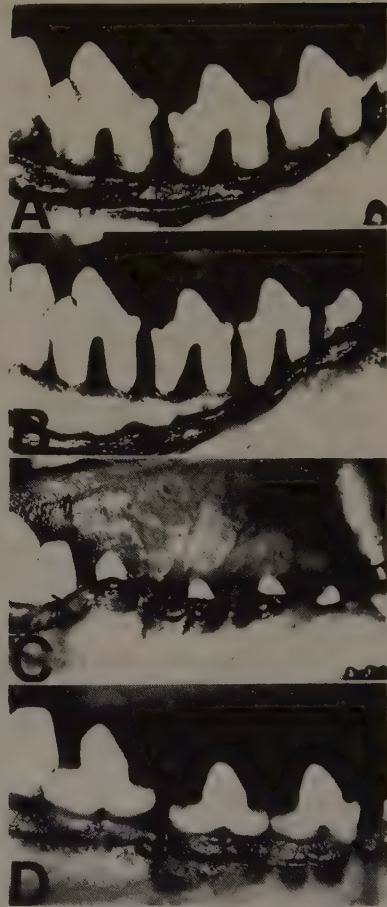


Fig. 8. Study 2. Dogs. Right mandibular premolars of a dog subjected to coronally positioned flap technique.

A. Pre-operative conditions immediately before reconstructive surgery.

B. During reconstructive surgery demonstrating the alveolar bone height. This dog initially had bone removed to a level 5 mm apical to the cemento-enamel junction (CEJ). At reconstructive surgery the bone level average 6.4 mm from the CEJ.

C. The flaps sutured in a coronal position.

D. 10 weeks post-operatively.

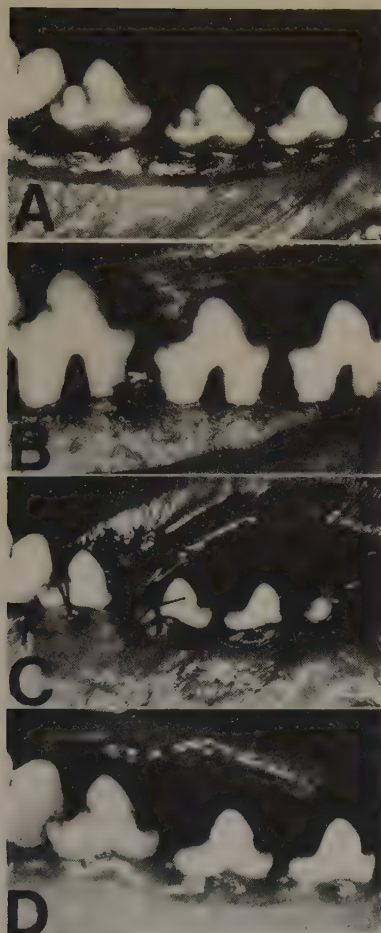


Fig. 9. Study 2. Dogs. Right mandibular premolars of a dog subjected to replaced flap technique.

A. Pre-operative conditions immediately before reconstructive surgery.

B. During reconstructive surgery demonstrating the alveolar bone height. This dog initially had bone removed to a level 3.5 mm apical to the CEJ.

C. The flaps replaced to the level of the CEJ.

D. 10 weeks post-operatively.

ical application of tetracycline ointment (Aureomycin® 3 %, Lederle, Great Britain) to the buccal and lingual tooth surfaces and gingival margins. In spite to this type of plaque control, some dogs started to develop plaque after six to eight weeks. The teeth with deposits were then polished and ointment applied as before. Plaque control was carried out under brief, light general pentothal anesthesia (Pentothal® Abbott Laboratories, North Chicago, Illinois, USA). Ten weeks after reconstructive surgery, block specimens consisting of tooth, bone and soft periodontal tissues were removed. The blocks were fixed in formalin, decalcified in EDTA, processed and evaluated as described in Study 1.

Results

Comparison of the clinical alveolar bone level measurements taken immediately after surgical bone reduction with the measurements at the time of reconstructive surgery 12 weeks later revealed that the alveolar bone had been further reduced. The amount of additional bone loss varied among the animals (Table 3).

The surgical therapy using coronally positioned flaps allowed for an average gingival coverage of the furcations of 4 mm immediately after suturing. The flap margins gradually moved apically and after one to two weeks the location of the gingival margins corresponded to the most coronal point of the furcations (Fig. 10). The replaced flaps allowed for an immediate average gingival coverage of 0.6 mm. These flap margins also shifted apically and after one week the gingival margins were generally positioned apical to the fornix of the furcations (Fig. 10).

The histological assessment of the healing showed that new attachment (complete & incomplete) was achieved in 12 out of the 18 teeth following the use of coronally posi-

Table 3

Study 2. Dogs. Clinical alveolar bone level (cemento-enamel junction to alveolar bone) at the time of reconstructive surgery related to the initial amount of surgical bone reduction. Means and ranges in mm for the six dogs under study

Dog #	Surgical alveolar bone reduction	Alveolar bone level at reconstructive surgery	Increase in alveolar bone reduction
1	2.0	2.6 (2.2-2.8)	0.6
2	2.0	3.5 (3.1-4.1)	1.5
3	3.5	3.5 (3.3-4.1)	0
4	3.5	4.1 (3.7-4.6)	0.6
5	5.0	5.5 (4.4-6.3)	0.5
6	5.0	6.4 (6.0-6.8)	1.4

tioned flap technique and in 1 out of 17 teeth following the replaced flap technique (Table 4).

Fig. 11 presents the histological healing response in relation to the defect size at the time of reconstructive surgery for teeth treated with the two flap techniques. It can be seen that new attachment occurred for teeth treated with coronally positioned flaps regardless of the defect size. Teeth treated with replaced flaps became epithelialized regardless of the preoperative defect size.

The mean interradicular bone level for the teeth with new attachment was 3.04 mm

coronal to the apical termination of root planing compared with 1.61 mm coronal for the teeth which became epithelialized.

Areas of ankylosis were observed in five of the 13 specimens which demonstrated new attachment (Fig. 12). Four of these specimens had large furcation defects (Fig. 11).

Discussion

Reconstructive periodontal surgery of experimentally induced furcation defects using citric acid conditioning of the instrumented

Table 4

Study 2. Dogs. Distribution of histological healing response. Number of teeth treated by coronally positioned and replaced flap techniques demonstrating complete new attachment, incomplete new attachment and epithelialized furcation

	Complete New Attachment	Incomplete New Attachment	Epithelialized Furcation
Coronally positioned flaps (N = 18)	3	9*	6
Replaced flaps (N = 17)	0	1	16

* 5 of these specimens showed ankylosis.

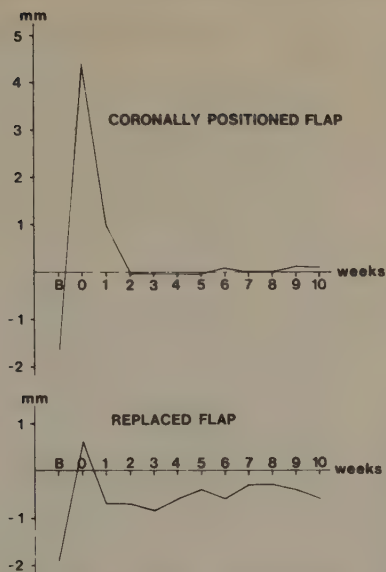


Fig. 10. Study 2. Dogs. Mean extent of gingival coverage of the furcations on the buccal aspects of teeth subjected to coronally positioned flaps and replaced flaps. Negative numbers denote a location of the gingival margin apical to the fornix of the furcation. B = before reconstructive surgery. 0 = immediately after suturing. 1, 2 etc. = weekly post-operative intervals.

root surfaces was performed in the present study in dogs. Two experimental variables were introduced: variation in placement and suturing of the flaps and variation in the size of the furcation defects.

The results indicate that adequate post-operative flap coverage of the furcations seems to be critical for successful healing of the furcation defects. New attachment and complete closure of the defects resulted in the majority of teeth subjected to the coronally positioned flap techniques. This was contrasted with an absence of new attachment following use of replaced flap techniques.

Variation of the vertical dimension of the defects appears to play a secondary role in the present dog model. Using the coronally positioned flaps, new attachment seemed to be achieved regardless of defect size. Creation of a smaller defect did not improve the success following replaced flap technique. The results of this study demonstrates the importance of adequate post-operative flap coverage over the furcation areas and the relative insignificance of the size of the furcation defects confirming the observations of the previous study in monkeys.

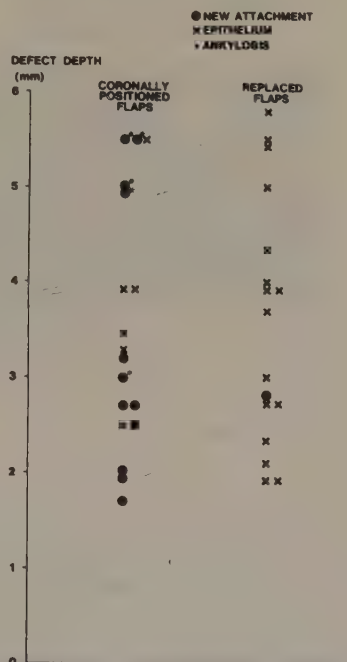


Fig. 11. Study 2. Dogs. Histological healing response (complete & incomplete new attachment versus epithelium) for teeth subjected to coronally positioned and replaced flap techniques in relation to defect depth. * = specimens demonstrating areas of ankylosis in the furcation fornix.

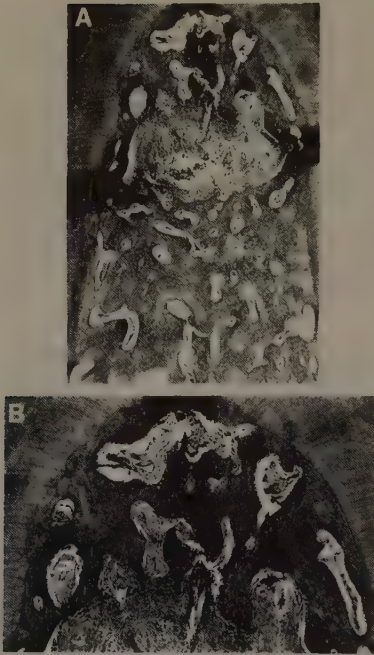


Fig. 12. Study 2. Dogs. A. Photomicrograph illustrating a specimen with extensive areas of ankylosis involving the full circumference of the furcation. The micrograph shows the top 2.9 mm portion of a defect which was 5.5 mm high at the time of reconstructive surgery.

B. Detail of the furcation fornix of A.

Comparing the two modes of flap placement, the clinical measurements of the position of the gingival margins show that the extent of furcation coverage was markedly different during the first two post-operative weeks. This observation is apparently related to the difference of healing response between the two flap techniques and seems to suggest that the first two post-operative weeks are critical in determining the final healing result and that complete flap cover-

age of the furcations during this period is essential.

The findings of the present study are seemingly different from the results of Nilvéus and Egelberg (1980), who obtained new attachment in dog furcation defects after the use of the replaced flap technique. In the study by Nilvéus and Egelberg (1980), however, alveolar bone reduction was limited to induction of "key-hole" furcation defects without concomitant horizontal bone reduction. The higher alveolar bone level proximal to the furcation seemed to give better post-operative support to the flaps and resulted in a gingival coverage of the furcations which was superior to that of the present study using the replaced flap technique. This difference in flap coverage between the two dog models most likely explains the discrepancy of healing results.

Areas of ankylosis were observed after healing of some of the largest furcation defects in both the present dog study as well as in the preceding monkey study. This is of interest in relation to the suggestion by Melcher (1976) that activity by cells originating from the pre-existing periodontal ligament may be a prerequisite for the regeneration of a new, normal periodontal ligament. Thus, it can be suggested that the healing of the coronal furcation areas in the large defects took place prior to the migration of periodontal ligament cells to these areas, thereby resulting in a disturbed healing pattern involving ankylosis.

In conclusion, adequate flap coverage of the furcation area seems to be critical for new attachment to occur following citric acid conditioning of the denuded furcations in the present dog model. Attempts at reconstructive surgery in furcation defects in man are, in our opinion, often hampered by difficulties in obtaining post-operative flap coverage. Future studies in experimental animals, therefore, should attempt to find methods that will provide for improved

post-operative flap coverage of the furcation area during treatment of human furcation involvements.

Acknowledgement

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Paper II

EFFECT OF PERIODIC TOOTH DISPLACEMENT ON
HEALING OF EXPERIMENTAL FURCATION
DEFECTS IN DOGS

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Key words: Periodontal regeneration - furcation defects -
tooth mobility.

Running head: Periodic tooth displacement

Abstract

This study compared the healing response, following reconstructive surgery, between teeth subjected to periodic displacement and teeth which were splinted during the postoperative healing period.

Through-and-through furcation defects were created in the mandibular second, third, and fourth premolars of 6 beagle dogs. Bone was surgically removed from the furcation and around each root to three different levels: 3, 6, and 9 mm. Reconstructive surgery included demineralization of the root surface and coronally positioned flaps. During the postoperative healing period the premolars on one side were subjected to periodic mesio-distal displacement by orthodontic elastics attached to splints. Corresponding teeth on the contralateral side were splinted to serve as non-mobile controls.

The results demonstrated that reattachment occurred in 12 of 14 displaced teeth and in 14 of 16 splinted teeth. There was no difference in the amount of interradicular bone fill or frequency of ankylosis between displaced and splinted teeth.

Our findings indicate that the healing response was not affected by tooth displacement in this dog furcation model.

The effect of tooth hypermobility on the results of periodontal therapy has been evaluated in human and animal studies. Rosling et al. (1976) reported that in treating a total of 124 2- or 3-wall infrabony defects all defects showed bone regeneration radiographically including 12 defects adjacent to teeth that maintained hypermobility at the end of the 24-month observation period. Similarly, Polson & Heijl (1978) treated 15 2-wall or 3-wall infrabony defects and observed bone fill at reentry 6-8 months postoperatively in all treated defects including 6 defects adjacent to teeth with persistent hypermobility.

Fleszar et al. (1980), however, in analysis of data from an 8 year longitudinal study involving 82 patients and 1974 teeth, found that the change in the probing attachment level following periodontal therapy was related to initial degrees of tooth mobility. Pockets associated with clinically hypermobile teeth did not respond to periodontal therapy as favorably, in terms of clinical attachment gain or loss, as did those associated with firm teeth. The reduced attachment levels of mobile teeth was observed for initially shallow, moderately deep and deep pockets. These clinical data are supported by animal experimentation by Lindhe & Ericsson (1976). They created infrabony defects on the mesial aspect of the mandibular fourth premolars in dogs and introduced jiggling type occlusal trauma. The periodontal pockets were surgically treated and occlusal trauma was continued in the experimental teeth but eliminated in control teeth. Histological analysis of 3-month biopsies showed that the bone crest of the test teeth was located more apically than the bone crest of the control teeth. Also, the apical termination of the junctional epithelium in the test teeth was located more apically than in the control teeth.

Thus, it appears that conflicting results have been obtained regarding the effect of tooth hypermobility on periodontal regeneration. The present study was initiated to further evaluate this problem. Through-and-through

furcation defects were created in mandibular premolars in dogs and the healing response in terms of connective tissue reattachment and bone formation was compared between teeth subjected to periodic displacement and teeth which were splinted during the postoperative healing period.

MATERIAL AND METHODS

Animals

6 female beagle dogs, 15-24 months old and weighing 9-12 kilograms were selected for the study. All surgery was carried out under balanced anesthesia using pethidin, diazepam and pentobarbitone (Green 1982). Atropine was administered preoperatively in order to reduce salivation. During surgery the animals were given 5 per cent Ringer's Glucose solution intravenously. The animals were fed a soft, canned dogfood diet throughout the study.

Periodic tooth displacement

During the postoperative healing period the mandibular second, third, and fourth premolars on one side (p2, p3, p4) were subjected to periodic displacement. Corresponding teeth on the contralateral side were splinted to serve as non-mobile controls. Displacement and splinting were alternated between the right and left sides in successive dogs.

Stainless steel onlays extending from the cuspid to the first molar were fabricated and cemented on both sides of the mandibular arch for each dog. The onlays were perforated by circular holes over the premolars under study exposing the cusp tips. These holes were adjusted to permit mesio-distal tooth displacement up to 2.5 mm on the experimental side.

A bucco-lingual groove was made in the flatened cusp tip of the experimental teeth. This groove was extended buccally and lingually in the occlusal surface of the splint to serve as a reference line for measurements of tooth displacement. Orthodontic elastics fastened to hooks on the splints were used to displace the teeth. The elastics could be shifted to allow for pull

of the experimental teeth mesially or distally.

The amount of tooth displacement was determined for each tooth by adding the mesial and distal displacements, i.e the displacements obtained following placement of elastics pulling the tooth in both mesial and distal directions. The displacement was recorded to the nearest 1/10 of a millimeter using sliding callipers. Measurements were made immediately after completion of reconstructive surgery and at the termination of the experiment.

On the control side, the splint was cemented to all teeth from the cuspid, to the first molar.

Experimental procedures

Through-and-through furcation defects were created in the mandibular second, third, and fourth premolars as described by Klinge et al. (1981). Bone was removed surgically from the furcations and from the full circumference of the teeth. 3 different levels of bone reduction were studied. In 2 dogs bone was removed to a level 3 mm apical to the cemento-enamel junction, 2 dogs had bone reduced 6 mm and another 2 dogs to a level 9 mm apical to the cemento-enamel junction.

Immediately following bone reduction the roots were planed and treated by topical citric acid application. Flaps were raised coronally and sutured to provide maximum coverage of the furcation wounds (Klinge et al. 1981).

The orthodontic elastics were shifted every day throughout the postoperative period, displacing the teeth alternately in a mesial or distal direction. Plaque control was also carried out daily by topical application of tetracycline ointment (Aureomycin 3%, Lederle, Great Britain) to the buccal and lingual tooth surfaces and gingival margins. These procedures were performed under brief, light thiopentone anesthesia. The position of the gingival margin in relation to the furcations was recorded weekly on the buccal aspects of the teeth as described by Klinge et al. (1981).

Histological procedures

9 weeks after surgery the dogs were sacrificed and block specimens consisting of tooth, bone and soft periodontal tissues were removed. The blocks were fixed in formalin, decalcified in formic acid, trimmed, and embedded in paraffin. Serial mesio-distal sections, 7 μ m thick, were cut throughout the entire bucco-lingual extension of the teeth. Every fourteenth section (at 98 μ m intervals) was stained with hematoxylin and eosin and examined using the light microscope.

The proportion of teeth with reattachment in the furcation area was determined from these stained step serial sections according to the following criteria:

complete reattachment: connective tissue regeneration including the formation of new cementum in the full circumference of the furcation in at least 3 consecutive stained sections.

incomplete reattachment: connective tissue regeneration in the furcation in at least 3 consecutive stained sections but lack of visible new cementum in part of the furcation or the presence of ankylosis.

epithelialized furcation: the presence of an epithelial lining in the furcation fornix or the absence of 3 consecutive stained sections showing complete or incomplete reattachment.

Histometrics were employed to determine: 1) the amount of interradicular bone fill, 2) the extent of interradicular ankylosis and 3) the size of the marginal periodontal ligament area of displaced and splinted teeth. Measurements were performed on 3 sections selected as follows: All stained step serial sections at 98 μ m intervals from each tooth were examined under x40 magnification. The distance between a horizontal line tangential to the most coronal interradicular bone and a horizontal line through the furcation

fornix was measured. The section with the shortest distance between these lines (=showing most bone apposition) and the adjacent step serial sections buccal and lingual to this section representing a span of 196 μm were selected. Means of measurements obtained on magnified tracings ($\times 125$) of these 3 sections were used to represent each tooth.

1) Interradicular bone fill:

First, the total furcation area coronal to a line through the apical termination of root planing for the mesial and distal roots was measured using a sonic digitizer (Grafbar, model GP-7, Science Accessories Corporation, Southport, CN, USA). The area of interradicular bone coronal to this line was measured, and the mean interradicular bone fill in percent of the total furcation area was computed.

2) Ankylosis:

The total linear circumference of the furcation coronal to the apical terminations of root planing for the mesial and distal roots was determined using an odometer. The proportion of this circumference demonstrating ankylosis of bone to root surface was calculated in percent.

3) Marginal periodontal ligament area:

The size of the marginal periodontal ligament area (Svanberg & Lindhe 1973) was determined for the proximal as well as the interradicular ligaments of both mesial and distal roots, as follows: A horizontal line was drawn across the width of the ligament at its most coronal point. A line parallel to the first line was drawn 2.0 mm apically, also across the width of the periodontal ligament. The surface area enclosed by these 2 horizontal lines, the root surface and the alveolar bone facing the ligament space was measured.

3 experimental and 2 control teeth were lost during histologic preparation. 1 experimental tooth did not show measureable displacement either immediately postoperatively or at 9 weeks and was therefore excluded for anal-

ysis. This left 14 experimental and 16 control teeth available for analysis.

Statistical analyses

The effect of tooth displacement versus splinting on 1) interradicular bone fill; 2) ankylosis; and 3) marginal periodontal ligament areas was evaluated for the 30 available specimens with analysis of variance using animal and tooth type as covariates.

RESULTS

The amount of flap coverage throughout the postoperative period relative to the fornices of the furcations is shown in Fig. 1. The average position of the gingival margin immediately after surgery was 2.2 mm coronal to the furcation fornix for displaced teeth and 2.9 mm for splinted teeth. Flaps retracted apically but still remained an average of 0.3 mm coronal to the furcations 9 weeks after surgery.

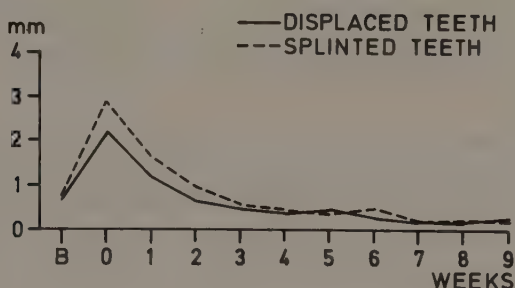


Fig. 1. Average position of the gingival margins relative to the furcation fornix for displaced and splinted teeth. B = before reconstructive surgery, 0 = immediately after suturing, 1,2, etc = weekly postoperative intervals.

The amount of tooth displacement for the experimental teeth is shown in Fig. 2. Displacement immediately after surgery varied from less than 0.1 mm to 1.4 mm. 9 weeks postoperatively displacement varied between 0.5 mm and 2.5 mm. The sizes of the marginal periodontal ligament areas are presented in Fig. 3. Statistical analyses revealed significant differences between displaced and splinted teeth for all 4 surfaces measured.

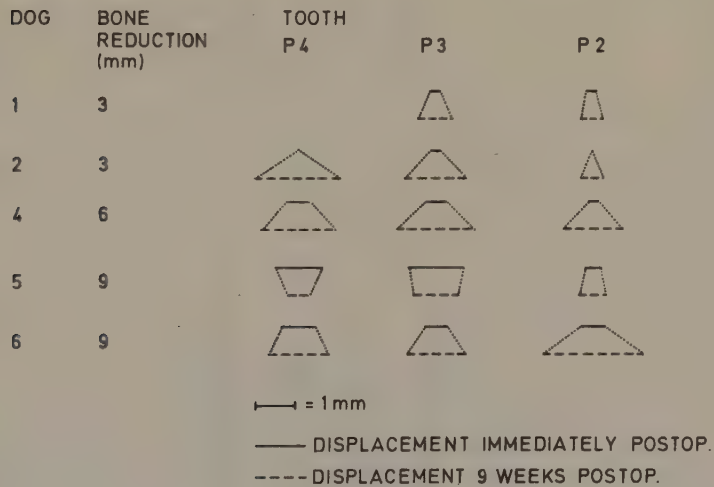


Fig. 2. Schematic illustration of clinical tooth displacement of experimental teeth immediately postoperatively and 9 weeks postoperatively for the individual dogs.

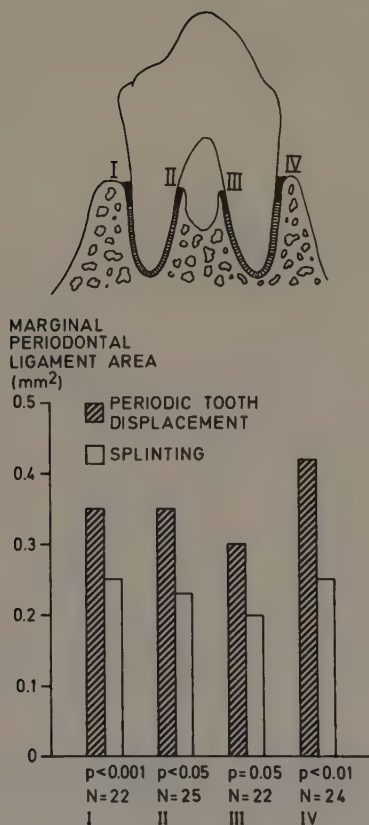


Fig. 3. Marginal periodontal ligament area following periodic tooth displacement and splinting by surface location (I, II, III, IV). N=number of surfaces available for measurements.

Reattachment (complete and incomplete) was observed in 12 of 14 displaced teeth and in 14 of 16 splinted teeth (Table 1). Mean interradicular bone fill was 31% for displaced teeth and 35% for splinted teeth. Ankylosis was observed in 10 of 14 displaced teeth and in 10 of 16 splinted teeth. The mean percentage of interradicular root surface with ankylosis amounted to 12% for displaced teeth and 15% for splinted teeth (Table 2). None of these measurements showed statistically significant differences between displaced and splinted teeth.

Table 1. Number of teeth demonstrating complete reattachment, incomplete reattachment and epithelialized furcations. N= number of available teeth

Defect size (mm)	Displaced teeth			Splinted teeth		
	3 (N=5)	6 (N=3)	9 (N=6)	3 (N=5)	6 (N=5)	9 (N=6)
Complete reattachment	0	0	0	3	0	0
Incomplete reattachment	4	3	5	2	5	4
Epithelialized furcation	1	0	1	0	0	2

Table 2. Interradicular bone fill and ankylosis for displaced and splinted teeth. Means and ranges

	Interradicular bone fill (%)	Ankylosis (%)
Displaced teeth (N=14)	31 (5-85)	12 (0-56)
Splinted teeth (N=16)	35 (8-64)	15 (0-49)

DISCUSSION

The effect of periodic tooth displacement on healing of through-and-through furcation defects in premolars in dogs was evaluated in the present study. No difference in healing was observed when comparing teeth subjected to daily displacement and teeth which were splinted during the postoperative healing period.

Various animal models have been utilized in the past to evaluate the impact of tooth hypermobility. Traumatic forces with alternating direction ("jiggling") were introduced by Wentz et al. (1958). They observed widened periodontal ligaments after 6 months of bucco-lingual jiggling of maxillary bicuspid in monkeys. A device producing jiggling type trauma in the mesio-distal direction in the fourth premolar of dogs was initiated by Svanberg & Lindhe (1973). Polson et al. (1976) simulated jiggling type trauma in the interproximal area between mandibular second and third bicuspid in monkeys by wedges alternatively placed in the interproximal area between third bicuspid and first molar and the interproximal area between first and second bicuspid. Every other day, the wedge was removed from one interproximal space and a new one placed in the other space. Through this method the interproximal periodontium between the second and third bicuspid was sub-

jected to repeated trauma by the alternate mesial and distal movement of these teeth.

In the present study periodic tooth displacement was induced by orthodontic elastics that were shifted daily and provided alternating pull of the experimental teeth mesially or distally. The splint prevented unintentional tooth displacement in bucco-lingual direction. The magnitude of the experimental bone reduction allowed some tooth displacement for most teeth immediately after reconstructive surgery. This necessitated splinting of the control teeth in order to obtain a non-mobile control.

The majority of experimental teeth demonstrated increased displacement 9 weeks postoperatively compared to immediately postoperatively. Measurements of the marginal periodontal ligaments at 9 weeks revealed larger ligament spaces for displaced teeth as compared to splinted teeth. This is in agreement with findings by Svanberg & Lindhe (1973) and Lindhe & Ericsson (1976). However, the relative increase in periodontal ligament area was less pronounced in our study as compared to Lindhe & Ericsson (1976). This difference could be explained by the fact that the tooth displacement in our experiment may have been of a smaller magnitude than that of Lindhe & Ericsson (1976). Also, it can not be excluded that the moderate tooth displacement in our study to some extent caused the bone surrounding the teeth to move with the teeth. Such a phenomenon would probably reduce the difference in marginal periodontal ligament area between displaced and splinted teeth.

3 different levels of bone reduction were studied in the present investigation. It could be expected that this would lead to varying degrees of tooth displacement. However, no difference was apparent in magnitude of 9-week displacement between teeth with different amounts of bone reduction. Furthermore, no difference in healing response was detected between teeth with different defect size regardless of displacement or splinting.

Klinge et al. (1981) showed new attachment in a majority of similar furcation defects, including large defects, (~6 mm), following acid conditioning of the instrumented root surface and coronal flap placement (mean furcation coverage 4.5 mm). In the present study healing was accomplished in even larger defects (~9 mm) using a limited coronal flap placement (mean 2.9 mm). It is possible that the magnitude of coronal flap positioning is of limited importance, provided a certain amount of coverage of the furcations is accomplished.

No difference in interradicular bone fill was seen comparing displaced and splinted teeth. This finding is in agreement with clinical reports by Rosling et al. (1976) and Polson & Heijl (1978). However, Lindhe & Ericsson (1976) found that the bone crest of teeth in which jiggling type occlusal trauma was continued following surgery was located more apically than the bone crest of the control teeth where the trauma was eliminated. Again, the difference in results between the present study and Lindhe & Ericsson (1976) may be explained by a difference in magnitude and frequency of tooth displacement.

The occurrence of ankylosis in the interradicular area was similar for displaced and splinted teeth. This finding seems at variance from observations of ankylosis comparing splinted and non-splinted teeth following tooth replantation. Andreasen (1975) used an extraoral period of 18 minutes following extraction and a healing period of 2 or 6 weeks following replantation found ankylosis and root resorption in the majority of splinted teeth but only in a few of the non-splinted teeth. Healing following replantation after an extraoral period of 120 minutes, however, showed ankylosis in all splinted and non-splinted teeth. Nasjleti et al. (1982) replanted monkey teeth after an extraoral period not exceeding 30 minutes. Splints were removed after 7 and 30 days. Replanted teeth that were splinted for 7 days showed less ankylosis and root resorption when compared to teeth splinted

for 30 days.

Ankylosis as a phenomenon following tooth replantation may be different in nature from ankylosis occurring during regeneration of marginal periodontal wounds. Therefore, it may not be pertinent to compare the incidence of ankylosis of splinted and non-splinted teeth following tooth replantation and following regeneration of marginal periodontal wounds.

In conclusion, periodontal healing of experimentally created dog furcation defects did not seem to be affected by tooth displacement following reconstructive surgery. The specificity of the model, both in terms of periodontal defects and tooth displacement, should be kept in mind and may not allow direct extrapolation to a clinical situation.

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Paper III

EFFECT OF IMPLANTS ON HEALING OF EXPERIMENTAL
FURCATION DEFECTS IN DOGS

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Key words: Periodontal regeneration - furcation defects -
implants.

Running head: Effect of implants

Abstract

The present study was performed to find out if implants placed around periodontally involved teeth during reconstructive surgery would support replaced flaps and prolong gingival coverage of the treated root surfaces facilitating new attachment.

Through-and-through furcation defects were made in the mandibular second, third, and fourth premolars of 10 beagle dogs. Bone was surgically removed from the furcation and from the circumference of each tooth to a level 3 to 4 mm apical to the cemento-enamel junction. Steel wires were passed through the furcations and ligated to enhance plaque formation. At 6 weeks, the wires were removed. At 12 weeks, during reconstructive surgery, 4 dogs received implants of autogenous intraoral cancellous bone, 2 of autogenous iliac cancellous bone and marrow, 2 of Proplast[®], and 2 of Avitene[®]. The results demonstrated that none of 12 teeth implanted with Avitene[®] or Proplast[®] showed new attachment. Only 2 of 6 teeth implanted with autogenous iliac bone showed new attachment, combined with extensive root resorption and ankylosis. Of 12 teeth implanted with autogenous intraoral bone, 6 showed new attachment. Our findings suggest that flap support by intraoral cancellous bone implants may facilitate new attachment. A more predictable technique, however, needs to be developed.

In through-and-through furcation defects in dogs, new connective tissue attachment has been achieved following reconstructive surgery with citric acid conditioning of the root surfaces (Crigger et al. 1978, Nilvéus et al. 1980). The amount of flap coverage following surgery seems to be important in obtaining successful results. New attachment has been achieved in a majority of large furcation defects treated with coronally positioned flaps, in contrast to the absence of new attachment in similar defects treated with replaced flaps (Klinge et al. 1981). With replaced flaps, a lack of underlying tissue support may allow the flaps to recede apical to the fornix of the furcation.

Extensive flap coverage through coronal positioning proved simple to obtain in dogs: a similar procedure would be more difficult in humans. Therefore, a series of studies was undertaken to determine if implants could support replaced flaps during healing and facilitate new attachment in dog furcation defects.

MATERIAL AND METHODS

Animals

10 female beagle dogs 18 to 22 months old and weighing between 9 to 14 kg were selected for the study. 4 dogs received implants of intraoral cancellous bone, 2 autogenous iliac cancellous bone and marrow, 2 Proplast[®], and 2 Avitene[®]. All surgery was carried out under balanced anesthesia using pethidine, diazepam, and pentobarbitone (Green 1982). Atropine sulfate was administered preoperatively to reduce salivation. During surgery, the dogs were given a 5 per cent Ringer's Glucose solution intravenously. The animals were fed a soft, canned, dogfood diet throughout the study.

Induction of defects

Through-and-through furcation defects were made in the mandibular second, third, and fourth premolars as described by Klinge et al. (1981). Bone was

surgically removed from the furcations and from the circumference of the teeth to a level 3 to 4 mm apical to the cemento-enamel junction. Steel wires were passed through the furcations and ligated to enhance plaque formation and prevent spontaneous healing. At 6 weeks, these wires were removed.

Experimental procedures

Reconstructive surgery was performed at 12 weeks. This procedure included reflecting mucoperiosteal flaps, debriding and planing the roots, and applying citric acid to the root surfaces. Teeth on 1 side of the mandible served as non-implanted controls. On the other side of the mandible implants were placed into furcations and interdental areas to the height and shape of the original alveolar bone as follows:

Autogenous intraoral cancellous bone grafts: 6 weeks prior to reconstructive surgery, the maxillary second and third premolars on both sides were extracted. Cancellous bone from the healing sockets of these teeth was removed during reconstructive surgery and, 1-3 mm pieces were immediately packed loosely into the furcations and interdental areas of the experimental teeth.

Autogenous iliac cancellous bone and marrow grafts: At the time of reconstructive surgery, cancellous bone and marrow was procured by an open cut-down of the superior iliac crest. The graft was stored in blood-soaked gauze until the recipient site was prepared, then 1-3 mm pieces of the graft were loosely packed into furcations and interdental areas.

Proplast[®]: Pieces of a polytetrafluorethylene carbon composite, Proplast[®] (Vitek Inc., Houston, Texas) were cut from a larger block: 1 piece was fitted in each furcation and 1 piece in each interdental area.

Avitene[®]: A microfibrillar collagen, Avitene[®] (Avicon Int., Fort Worth, Texas) was loosely packed into furcations and interdental areas.

After the implants were placed, the flaps were replaced and sutured with single interdental sutures. The flaps on the control side of each jaw were also replaced and sutured interdentally.

The position of the gingival margin relative to the fornix of the furcations on the buccal aspects of the teeth was recorded throughout the study as described by Klinge et al. (1981).

Plaque control consisted of tetracycline ointment (Aureomycin 3% Lederle, Great Britain) applied topically every other day to the buccal and lingual tooth surfaces and gingival margins under brief, light, thiopentone anesthesia.

Histological procedures

6 weeks after surgery, the dogs were sacrificed, and specimen blocks consisting of tooth, bone, and soft periodontal tissues were removed. The blocks were fixed in formalin, decalcified in formic acid, trimmed, and embedded in paraffin. Serial mesiodistal sections, 7 μ m thick, were cut throughout the entire buccolingual extension of the teeth. Every fourteenth section was stained with hematoxylin and eosin and examined using the light microscope. Each tooth was categorized according to the following criteria:

complete new attachment: connective tissue regeneration including the formation of new cementum in the full circumference of the furcation in at least 3 consecutive stained sections.

incomplete new attachment: connective tissue regeneration in the furcation in at least 3 consecutive stained sections but lack of visible new cementum in part of the furcation or the presence of ankylosis in any of the 3 sections.

epithelialized furcation: the presence of an epithelial lining in the furcation fornix or the absence of 3 consecutive stained sections showing complete or incomplete new attachment.

RESULTS

The amount of flap coverage throughout the postoperative period relative to the fornices of the furcations is shown in Fig. 1. The average gingival coverage was 0.8 mm immediately after surgery for both implanted and control teeth. The flap margins gradually receded, and after a few weeks, were generally at or below the fornix. At teeth treated with implants the gingival margins tended to recede less than at the control teeth.

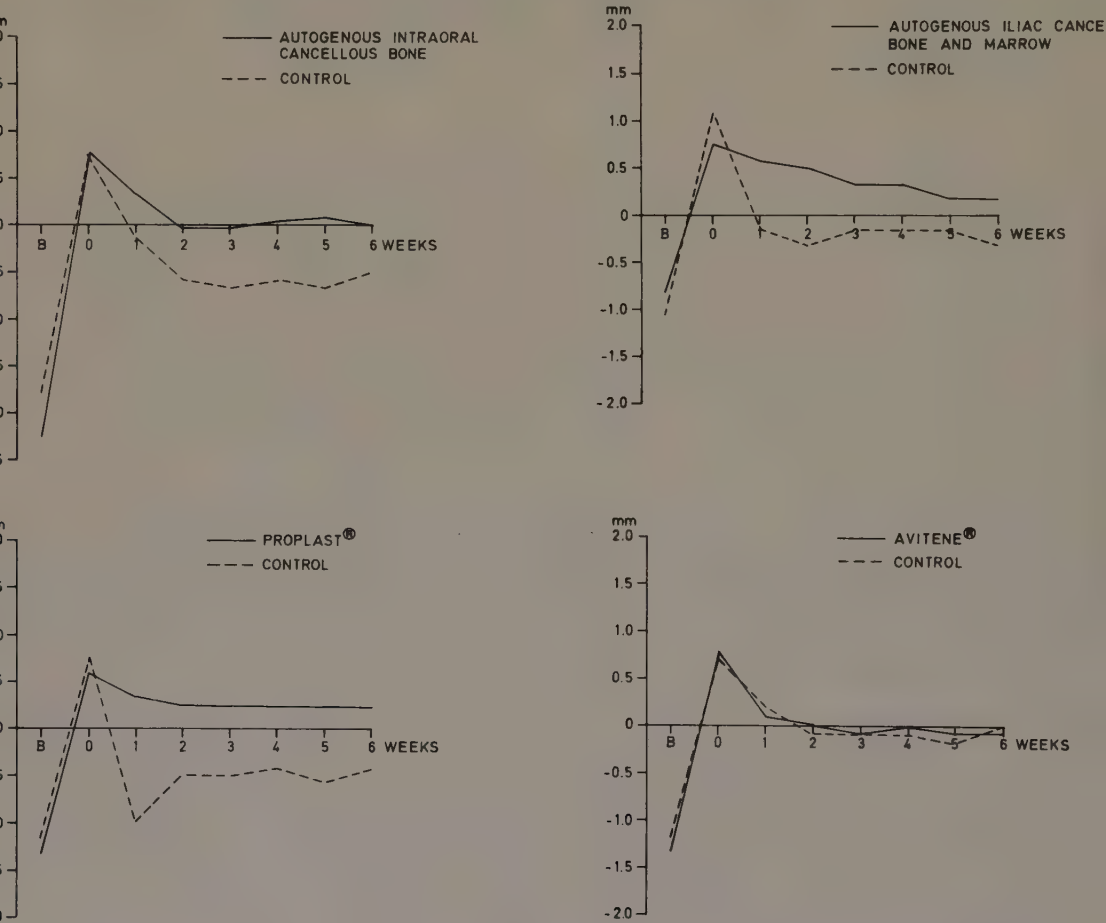


Fig. 1. Position of gingival margin relative to the furcation fornix for the 4 different types of implants used: autogenous intraoral cancellous bone, autogenous iliac cancellous bone and marrow, Proplast®, Avitene®. B = before reconstructive surgery, 0 = immediately after suturing, 1,2, etc = weekly postoperative intervals.

The results of the histological assessment are shown in Table 1. New attachment was observed in 6 of 12 teeth implanted with intraoral cancellous bone and in 2 of 6 teeth implanted with iliac bone; these 2 teeth also exhibited extensive root resorption and ankylosis. None of the 12 teeth implanted with Proplast® or Avitene® showed new attachment. Of the 29 teeth not receiving implants, 28 became epithelialized.

Table 1. Number of teeth demonstrating complete new attachment, incomplete new attachment or epithelialized furcations by different implants.

Implant	Complete new attachment	Incomplete new attachment	Epithelialized furcation
Autogenous intraoral cancellous bone (N=12)	3	3	6
Autogenous iliac can- cellous bone and marrow (N=6)	0	2	4
Proplast® (N=6)	0	0	6
Avitene® (N=6)	0	0	6
Non implanted control teeth (N=29) ^x	0	1	28

^x 1 specimen extracted prior to reconstructive surgery due to fused roots.

Fig. 2 compares the postoperative flap coverage for the teeth implanted with intraoral cancellous bone grafts with and without new attachment. The gingival margin in teeth with new attachment remained above the fornix throughout the healing period, in contrast to epithelialized teeth in which the gingival margin receded apical to the fornix at 2 weeks.

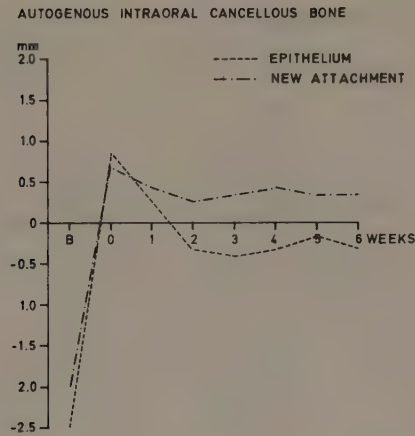


Fig. 2. Position of gingival margin relative to the furcation fornix for teeth implanted with intraoral cancellous bone separated into teeth that healed with new attachment versus teeth showing epithelium.

DISCUSSION

Our previous studies indicate that citric acid conditioning and coronally positioned flaps are necessary procedures to reconstructive surgery for new attachment to occur in large, through-and-through furcation defects in dogs (Klinge et al. 1981). However, the type of coronally positioned flaps used in dogs have limited clinical application in humans. Flaps replaced at the approximate level of the cemento-enamel junction recede early in the healing process, exposing the fornix of the furcation and thus counteract the formation of new attachment (Klinge et al. 1981).

In this study attempts were made to maintain the margins of replaced flaps at their immediate postsurgical levels during healing by using supportive implants under the flaps. The results indicate that successful healing with new attachment, when it occurred, was associated with maintained flap coverage.

The potential of new attachment following grafting with autogenous intraoral cancellous bone in animals has been described by Ellegaard et al. (1974, 1975) and Nilv  us et al. (1978) and in human intrabony defects by Bowers et al. (1982). This potential was confirmed in our study where new attachment was achieved in 6 of 12 teeth implanted with autogenous intraoral cancellous bone. The new attachment was achieved only in teeth where the flap margin remained coronal to the fornix of the furcation throughout the postoperative period. However, it is uncertain whether healing caused the flaps to remain in position or if the postoperative flap coverage initiated healing.

Autogenous iliac cancellous bone and marrow has a high potential for osteogenesis when implanted in experimental intrabony pockets or furcation defects in animals (Ellegaard et al. 1973, 1974, 1976). However, healing is usually accompanied by resorption and ankylosis of the roots. In our

study, 2 of 6 experimental teeth showed new attachment with extensive root resorption and ankylosis.

Proplast[®] (polytetrafluorethylene carbon composite) has been used in reconstructive orthopedic and maxillofacial surgery. Connective tissue growth and mineralization without contemporary inflammatory reactions in this material have been shown among others by Kent et al. (1975) and by Halstead et al. (1979).

Collagen and collagen derivatives have been used extensively in sutures and in fascia and blood vessel prostheses. Avitene[®] is a microfibrillar collagen, derived from animal hide, that causes only minimal foreign body reactions, and carries a little risk of causing adverse immunologic reactions (Zeleznick 1975).

New attachment was not associated with Proplast[®] or Avitene[®]; these implants may not have been incorporated into the healing process in the furcation prior to epithelialization.

In spite of the limited number of teeth studied, our results indicate that intraoral cancellous bone implants support replaced flaps and may favour new attachment in this animal model. However, new attachment was not a consistent finding and more predictable techniques should be developed.

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Paper IV

EFFECT OF CROWN-ATTACHED SUTURES
ON HEALING OF EXPERIMENTAL
FURCATION DEFECTS IN DOGS

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Key words: Periodontal regeneration - furcation
defects.

Running head: Crown-attached sutures

Abstract

This study evaluates if suturing of replaced flaps with crown-attached sutures, following reconstructive surgery, counteracts postoperative recession of the gingival margins and facilitate new attachment.

Through-and-through furcation defects were created in the mandibular premolars of 5 beagle dogs. Bone was surgically removed from the furcation and around each root to a level 3 to 4 mm below the cemento-enamel junction. Steel wires were placed in the furcations for 6 weeks to enhance plaque formation. After 12 weeks, reconstructive surgery that included acid conditioning of root surfaces was performed. On 1 side of the mandible, the flaps were replaced and secured by bonding the sutures to the teeth with composite resin. On the other side, the replaced flaps were sutured interdentally with single sutures. The results demonstrated that the margins of flaps secured with interdental sutures shifted apically to the fornix of the furcations after 1 week, and histologically all 10 furcations were found to be lined with epithelium. Margins of flaps secured with crown-attached sutures remained an average of 0.5 mm coronally to the fornix, and 13 of the 14 teeth showed new attachment.

The results indicate that a suturing technique which counteract gingival recession, favours the formation of new attachment in this beagle dog model.

New connective tissue attachment in through-and-through furcation defects in dogs is favoured by conditioning debrided and planed root surfaces with citric acid (Crigger et al. 1978, Nilvéus et al. 1980) and by creating initial, extensive flap coverage of the furcations (Klinge et al. 1981). The type of extent of coronally positioned flaps which results in new attachment in dogs is clinically inapplicable in humans.

Flaps replaced slightly above the cemento-enamel junction recede and the gingival margins are located apically to the fornix of the furcations within 1 week after surgery: new attachment has not been achieved with replaced flaps (Klinge et al. 1981). Attempts at providing flap support by various implants have shown limited success (Klinge et al. 1984). The present study was undertaken to test if a suturing technique aiming at prolonged coverage of the furcation region by replaced flaps favours the development of new attachment.

MATERIAL AND METHODS

Animals

5 female beagle dogs 18 to 24 months old and weighing 12 to 16 kg were selected. All surgery was carried out under balanced anaesthesia using pethidin, diazepam and pentobarbitone (Green 1982). Atropine sulfate was administered preoperatively in order to reduce salivation. During surgery, the dogs were given a 5 per cent Ringer's Glucose solution intravenously. The animals were fed a soft, canned dogfood diet throughout the study.

Induction of defects

Through-and-through furcation defects were made in the mandibular second, third, and fourth premolars as described by Klinge et al. (1981). In short, bone was removed surgically from the furcations and from the circumference of the teeth to a level 3 to 4 mm apical to the cemento-enamel junction. Steel wires were passed through the furcations and ligated to enhance plaque

formation and prevent spontaneous healing. After 6 weeks, these wires were removed.

Experimental procedures

Reconstructive surgery was performed at 12 weeks. This procedure included elevation of mucoperiosteal flaps, debridement and planing of the roots, as well as application of citric acid to the root surfaces. On 1 side of the mandible, the flaps were replaced and secured with crown-attached sutures, which were bonded to acid etched enamel with a composite resin (Fig. 1).



Fig. 1. Sequence of suturing using crown-attached sutures:

- 1) a single interdental suture is tied and the tags are left untrimmed;
- 2) the suture is wrapped around an adjacent crown;
- 3) the suture is tied and bonded to acid etched enamel with a composite resin.

As a control, on the contralateral side, the replaced flaps were sutured interdentally with single sutures. Each technique was alternated between the right and left sides on successive dogs.

The position of the gingival margin relative to the fornix of the furcation on the buccal aspect of the teeth was recorded throughout the study as described by Klinge et al. (1981).

Plaque control was carried out by topical application of tetracycline

ointment (Aureomycin 3%, Lederle, Great Britain) every other day to the buccal and lingual tooth surfaces and gingival margins under brief, light, thiopentone anesthesia throughout the postoperative period.

Histological procedures

6 weeks after surgery, the dogs were sacrificed, and specimen blocks consisting of tooth, bone, and soft periodontal tissues were removed. The blocks were fixed in formalin, decalcified in formic acid, trimmed, and embedded in paraffin. Serial sections, 7 μ m thick, were cut in the mesiodistal plane throughout the entire bucco-lingual extension of the teeth. Every fourteenth section was stained with hematoxylin and eosin and examined using the light microscope. Each tooth was categorized according to the following criteria:

complete new attachment: connective tissue regeneration including the formation of new cementum in the full circumference of the furcation in at least 3 consecutive stained sections.

incomplete new attachment: connective tissue regeneration in the furcation in at least 3 consecutive stained sections but lack of visible new cementum in part of the furcation or the presence of ankylosis in any of the 3 sections.

epithelialized furcation: the presence of an epithelial lining in the furcation fornix or the absence of 3 consecutive stained sections showing complete or incomplete new attachment.

RESULTS

The amount of flap coverage throughout the postoperative period relative to the fornices of the furcations is shown in Fig. 2.

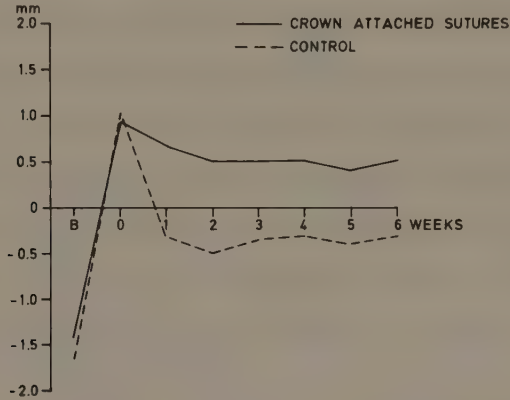


Fig. 2. Position of gingival margin relative to the furcation fornix by method of suturing. B= before reconstructive surgery, 0= immediately after suturing, 1,2, etc= weekly postoperative intervals.

The average position of the gingival margin immediately after surgery was 0.9 mm above the fornix in both crown-attached and single-sutured groups. Flaps secured with crown-attached sutures retracted apically an average of 0.4 mm but still remained an average of 0.5 mm coronally to the furcations 6 weeks after surgery. Flaps secured with interdental sutures retracted apically to below the fornix of the furcation within 1 week after surgery.

The histologic healing results are shown in Table 1. Among the teeth with the crown-attached sutures, all but one showed new attachment. In contrast, none of the teeth with interdental sutures showed new attachment: all had epithelialized furcations.

Table 1. Number of teeth demonstrating complete new attachment, incomplete new attachment or epithelialized furcations by methods of suturing

Method of suturing	Complete new attachment	Incomplete new attachment	Epithelialized furcation
Crown attached sutures (N=14) ^x	7	6	1
Single inter-dental sutures (N=10) ^{xx}	0	0	10

^x 1 tooth had fused roots and was extracted prior to reconstructive surgery

^{xx} 5 teeth were lost prior to reconstructive surgery due to extensive mobility

DISCUSSION

Postoperative coverage of furcations in the present dog model can be achieved with coronal flap positioning (Klinge et al. 1981) or, as demonstrated in this study, with replaced flaps using crown-attached sutures. We found that replaced flaps secured with crown-attached sutures are associated with new connective tissue attachment. The crown-attached sutures give less immediate coverage to the furcations than that with coronally positioned flaps (Klinge et al. 1981). The healing response here, however, indicates that the coverage achieved with crown-attached sutures is sufficient for new attachment to occur.

The initial flap coverage of the furcation after surgery was the same for both crown-attached and single-sutured groups. Replaced flaps secured with single interdental sutures rapidly receded below the fornix of the furcation. The lack of a similar recession with crown-attached sutures, coupled with the successful healing for this group indicates that new attach-

ment is achieved as a result of preventing flap retraction to below the for-nix.

The type of coronally positioned flaps that resulted in new attachment in dogs in our previous work (Klinge et al. 1981) is clinically inapplicable in humans. The results of this study indicates that crown-attached sutures combined with replaced flaps favours new attachment. Crown-attached sutures may be clinically applicable in humans.

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Paper V

BONE REGENERATION PATTERN AND ANKYLOSIS
IN EXPERIMENTAL FURCATION DEFECTS IN DOGS

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Key words: Furcation defects, bone regeneration,
ankylosis

Running head: Bone regeneration pattern and
ankylosis

Abstract

This study reports on the pattern of interradicular bone formation and the frequency and localization of ankylosis following regenerative surgery of experimentally induced furcation defects in 12 beagle dogs. Observations were made in 39 specimens showing healing with connective tissue attachment in the full circumference of the furcation. The findings indicate that bone regeneration first occurs adjacent to the root surfaces, followed by a subsequent fill of the central portion of the interradicular area. Ankylosis, observed in 24 of the 39 specimens, was more frequent in large defects than in small defects. The incidence of ankylosis increased with increased distance from the base of the original wound. These findings suggest that coronal bone apposition may require or be facilitated by the presence of a root surface, and that undisturbed coronal regeneration may be dependent upon cellular or some other activity from the intact periodontal ligament.

In our previous studies on healing of experimental furcation defects we observed that bone apposition often had progressed more coronally adjacent to the root surfaces as compared to the central part of the interradicular lesion (Crigger et al. 1978, Nilvéus & Egelberg 1980). In some areas, the newly formed bone was ankylosed to the root surface, especially in furcation defects with a large vertical dimension (Nilvéus et al. 1980, Klinge et al. 1981). Recent interest has been focused on the role of the root surface and the periodontal ligament cells in promoting undisturbed regeneration of periodontal tissues (Karring et al. 1980, Nyman et al. 1980, Boyko et al. 1981, Nyman et al. 1982a, Nyman et al. 1982b, Aukhill et al. 1983). The present study on bone formation pattern and frequency and localization of ankylosis in regenerating furcation defects in dogs is reported in the context of this interest.

MATERIAL AND METHODS

The material for the present report originates from 2 previous studies (study I: Klinge et al. 1981, study II: Klinge et al. 1984). It includes observations on 12 beagle dogs subjected to regenerative surgery in experimentally created furcation defects. Only specimens showing regeneration with reattachment in the full circumference of the furcation are included (39 of 65 treated teeth, 13 from study I and 26 from study II).

In short, through-and-through furcation defects were created in the mandibular second, third, and fourth premolars. Bone was removed surgically from the interradicular areas and from the circumference of the teeth to a level 2-5 mm (study I) and 3-9 mm (study II) apical to the cemento-enamel junction.

In study I steel wires were passed through the interradicular areas and ligated to enhance plaque accumulation. After 6 weeks, these wires were removed. Reconstructive surgery was performed at 12 weeks. In study II recon-

structive surgery was performed immediately following the bone reduction procedure. In this later study, as part of the experimental design, some teeth were subjected to periodic displacement, others were splinted following surgery.

Reconstructive surgery for both groups included elevation of mucoperiosteal flaps, debridement and planing of the roots as well as application of citric acid to the root surfaces. 2 types of wound closure were used. 1. Coronally positioned flaps: horizontal relaxing incisions were made through the periosteum on the inner aspects of the reflected flaps to allow for their coronal placement and suturing and to provide maximum coverage of the furcation wounds (38 of 48 treated teeth without the presence of an epithelial lining was included in this report). 2. Replaced flaps: the flaps were sutured with their margins at the approximate level of the cemento-enamel junction (1 of 17 treated teeth without the presence of an epithelial lining was included).

Plaque control was carried out by topical application of tetracycline ointment throughout the postoperative period.

Histological procedures

10 weeks (study I) or 9 weeks (study II) after surgery, the dogs were sacrificed, and specimen block consisting of tooth, bone and soft periodontal tissues were removed and processed for light microscopy. The healing response of the specimens was evaluated from mesio-distal, step serial sections at 98 μ m intervals.

Histometrics were employed to determine: 1) the pattern of interradicular bone formation; 2) the frequency and localization of ankylosis; and 3) the interradicular defect depth. Measurements were performed on 3 sections selected as follows: all step serial sections at 98 μ m intervals from each tooth were examined under x40 magnification. The distance between a horizontal line tangential to the most coronal interradicular bone and a horizontal

line through the furcation fornix was measured. The section with the shortest distance between these lines (= showing most bone apposition) and the adjacent step serial sections buccal and lingual to this section representing a span of 196 μm were selected. Using microscope projection these sections were magnified (x35) and their images depicted.

1) Bone formation pattern:

The height of regenerated bone was measured for 3 locations in the interradi-
cular area:

A) the vertical distance from the crest of the bone adjacent to the mesial root to the apical termination of root planing for this root,

B) the same distance for the distal root,

C) the height of a vertical line from the most coronal bone in the middle of the interradi-
cular areas to the intersection of this line with a line drawn between the apical terminations of root planing of mesial and distal roots.

By subtracting the distance C from distance A and distance B respectively, the advancement of the bone formation adjacent to the mesial and distal root surfaces relative to the middle interradi-
cular area could be evaluated. Means of measurements for the 3 selected sections were used to represent each tooth (Fig. 5).

2) Ankylosis:

The presence or absence of bone in direct apposition to the root surface was evaluated for each millimeter increment of the mesial and distal root surfaces coronal to apical termination of root planing. The incidence of anky-
losis within each millimeter increment (1st, 2nd, 3rd etc) was calculated in percent relative to the presence of bone within this increment. Thus, the frequency of ankylosis following bone apposition of increasing heights was determined. Calculations were based on all available 117 sections (3 sec-

tions x 39 specimens).

3) Defect depth:

The vertical distances between the furcation fornix and the apical termination of root planing for the mesial and distal root surfaces were measured. The average of the mesial and distal measurements for the 3 sections was used to compute the mean defect depth of each tooth (Klinge et al. 1981).

RESULTS

The extent of interradicular bone regeneration varied from limited amounts to bone filling the entire interradicular area (Figs. 1-4). Typically, the bone formation was more advanced adjacent to the root surfaces than in the midportion of the interradicular area, creating a U-shaped pattern of the bone (Fig. 1). This pattern was confirmed in the histometric evaluations (Fig. 5).

Ankylosis was observed in 24 of the 39 specimens. It was more frequent in large defects than in small defects: 13 of 14 defects ≥ 4.3 mm in vertical depth showed ankylosis as contrasted to 11 of 25 defects < 4.3 mm (Fig. 6). The incidence of ankylosis increased as bone was forming more coronally: in the 1st mm increment coronal to the apical termination of root planing only 5% of the surfaces with newly formed bone showed ankylosis. Bone formed in the 5th and 6th mm increments demonstrated around 90% frequency of ankylosis (Table 1).

Subgroup comparisons of specimens from study I and study II and specimens of study II subjected to displacement or splinting using analysis of variance did not demonstrate statistically significant differences with respect to either bone formation pattern or frequency of ankylosis.

Small areas of root resorption were often observed. The location of these resorptions varied. Some were found at the proximity of the apical termination of root planing in the root planed dentin or in the non-instrumented

old cementum. Resorptions were also observed at any location around the circumference of the furcation. Some of these resorptions were partially or totally filled with a cementum-like material, others were filled with bone ankylosed to the root surface, and others did not show any mineralized deposit but did present an occasional multinucleated, clast-like cell in the resorptive bay (Figs. 3a, 3b, 4a).



Fig. 1. Photomicrograph showing connective tissue attachment in furcation lesion. Original defect size = 3.7 mm. Bone formation is more advanced adjacent to the root surfaces than in the midportion of the interradicular area. Arrows indicate apical termination of root planing.

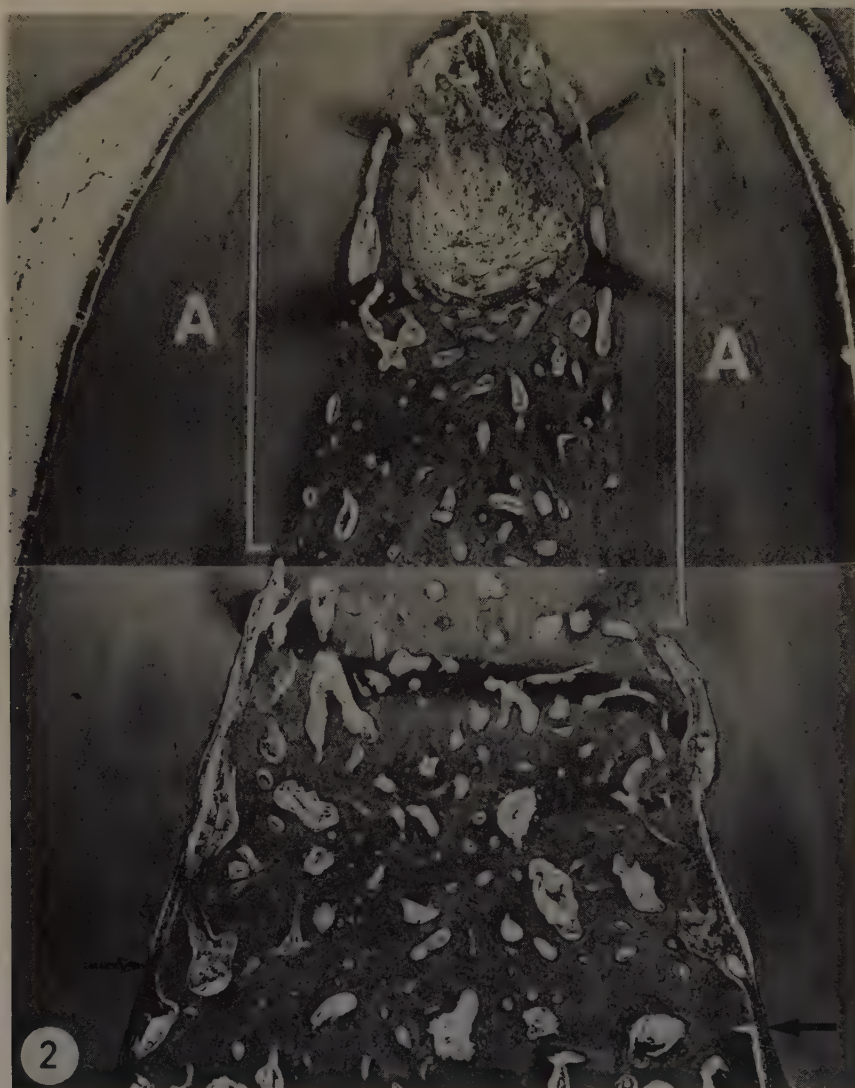


Fig. 2. Photomicrograph showing connective tissue attachment in furcation lesion. Original defect depth = 6.4 mm. Ankylosis (A) is present in the 2nd - 3rd mm increment coronal to the arrows indicating apical termination of root planing.

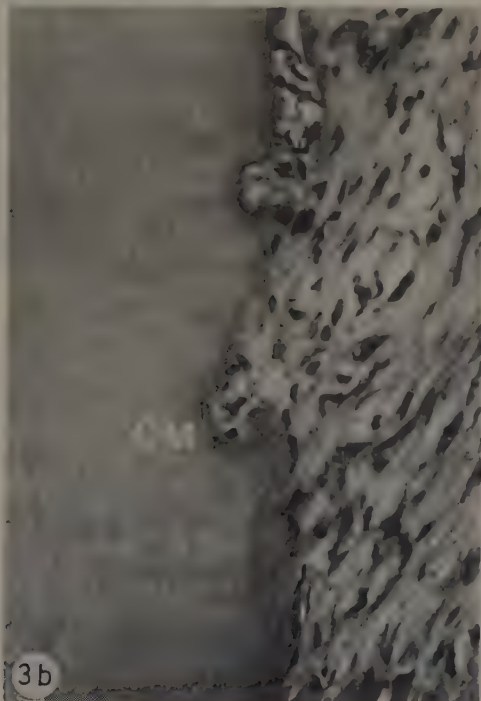
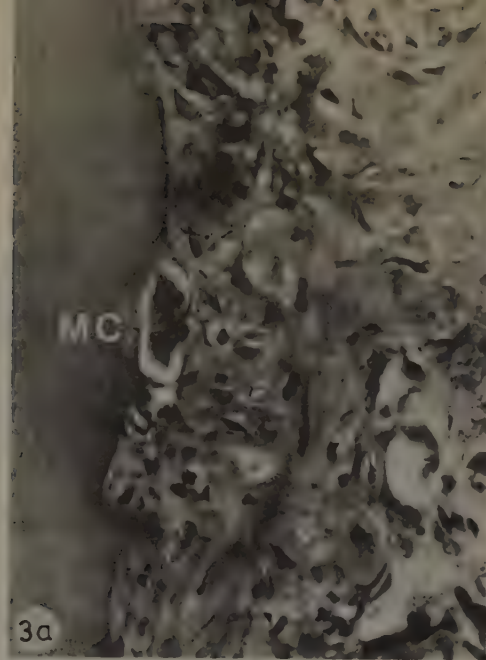


Fig. 3. Photomicrograph showing connective tissue attachment in furcation lesion. Original defect depth = 4.1 mm. Arrows indicate apical termination of root planing. Inserts indicate outline of high-power fields (Figs. 3a, 3b).

Fig. 3a. Resorption with a multinucleated cell (MC) in one of the resorption bays.

Fig. 3b. Resorptions partially filled with a cementum-like material (CM).



Fig. 4. Photomicrograph showing connective tissue attachment in furcation lesion. Original defect depth = 6.1 mm. Ankylosis (A) and resorptions (R) can be seen along the root surfaces. Insert indicate outline of high-power field (Fig. 4a). Arrows indicate apical termination of root planing. Fig. 4a. Bone ankylosed to the root surface (A).

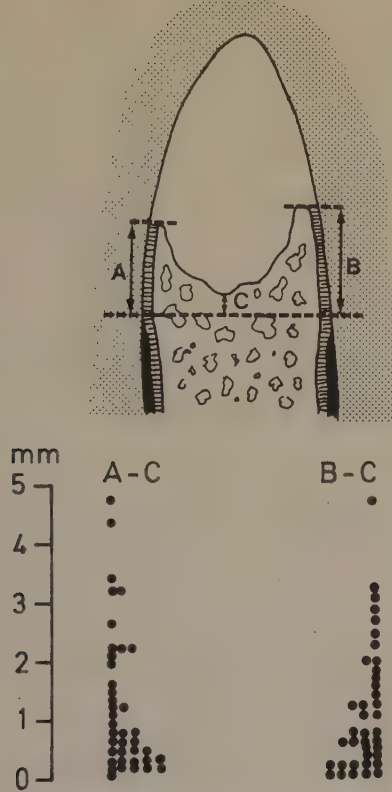


Fig. 5. Schematic illustration of coronal regeneration of interradicular bone. The diagram shows results from histometric analysis of the advancement of the bone formation adjacent to the mesial (A) and distal (B) root surfaces relative to the middle interradicular area (C).

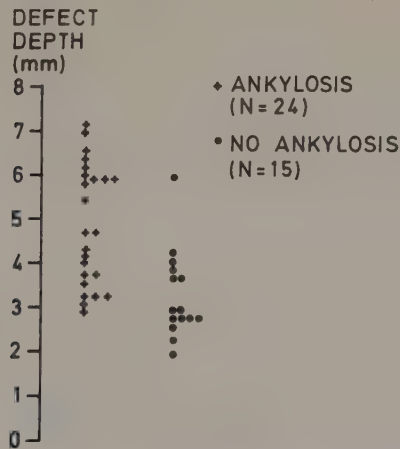


Fig. 6. Distribution of teeth with and without ankylosis related to defect depth. N=number of teeth available.

Table 1. Frequency of ankylosis in bone formed at various distances
(mm increments) from the apical termination of root planing

mm increment from apical ter- mination of root planing	frequency of ankylosis (%)
≤ 1.0	5
1.1 - 2.0	20
2.1 - 3.0	33
3.1 - 4.0	53
4.1 - 5.0	71
5.1 - 6.0	97
≥ 6.1	87

DISCUSSION

This cross sectional study of specimens obtained 9-10 weeks following regenerative surgery of experimental through-and-through furcation defects indicates that bone regeneration first occurs adjacent to the root surfaces, followed by a subsequent fill of the central portion of the interradicular area. This phenomenon suggests that coronal bone apposition may require or be facilitated by the presence of a root surface. Alternatively, it may indicate that coronal bone growth is dependent upon either cellular or some other activity originating in the periodontal ligament.

Acid root conditioning was performed as part of the regenerative surgery in the present study. This procedure exposes collagen fibrils on the dentin surfaces and prepares for the attachment of connective tissue to the root (Garrett et al. 1978, Ririe et al. 1980, Polson & Proye 1983). Acid conditioning seems to provide a hospitable surface for critical biochemical

and cellular events occurring in the coagulum immediately following wound closure (Boyko et al. 1980, Boyko et al. 1981, Fernyhough & Page 1983, Polson & Proye 1983). Therefore, it may enhance a subsequent coronal migration of blood vessel endothelium and progenitor cells for bone and ligament formation.

Melcher (1976) suggested that regeneration of periodontal ligament does not appear to be as rapid as that of alveolar bone. The findings of the present study support this suggestion. During coronal regeneration of the interradicular tissues, the periodontal ligament space had frequently been obliterated by ankylosis of bone to the root surface. The incidence of ankylosis increased with increased distance from the base of the original wound. Thus, it can be suggested that ligament progenitor cells migrating coronally and forming a periodontal ligament are "overrun" by bone forming cells and that this occurs more readily as the distance from the base of the wound increases.

Minor areas of root resorption were often observed, a few of them harboring multinucleated, clast-like cells. The majority of these resorptions showed "repair" with a cementum-like substance or with bone ankylosed to the root surface. This repair, however, may be temporary. It is possible that these small areas of resorption observed 9-10 weeks postoperatively continue to develop and may cause extensive replacement of root structure at later time points (Bogle et al. 1984, Magnusson et al. 1984).

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